1232823

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

September 30, 2004

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *60/485,073*
FILING DATE: *July 03, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/21451*

Certified by

Jon W Dudas

Acting Under Secretary of Commerce
for Intellectual Property
and Acting Director of the U.S.
Patent and Trademark Office

EXPRESSION OF A RECOMBINANT TRANSGENE

by

Richard Allison

Michigan State University ID number 03-012

Harness, Dickey & Pierce, P.L.C. Docket Number 6550-000072

FIELD

[0001] The present invention relates generally to virology, in particular to the use of a positive strand RNA virus to express a heterologous polypeptide in a transgenic host cell.

BACKGROUND

[0002] A transgene construct for expressing a heterologous polypeptide in a host cell, seed or organism usually comprises a promoter operably linked to a nucleic acid encoding a heterologous polypeptide. However, transgene expression can be toxic to the host cell, seed or organism, or inhibit growth of the host cell, seed or organism. Furthermore, in the case of transgenic plants, transgenic crop seed can contaminate non-transgenic seed, causing the appearance of an unwanted heterologous polypeptide in a crop. One solution to such problems has been to provide transgene constructs comprising an inducible promoter. In a host cell, cell culture, or organism harboring a transgene comprising an inducible promoter controlling a transgene, expression of the transgene can be delayed until the host cell, cell culture, or organism reaches a predetermined condition or stage of development, size, or cell density. Transcription of the transgene is then induced using a stimulus appropriate for the promoter. However, a significant problem with the use of an inducible promoter to direct transgene expression is that an inducible promoter can support background levels of transgene transcription (i.e., are "leaky") in the absence of the stimulus. Thus, the host cell comprises RNA coding for the heterologous polypeptide, even in the abscence of the stimulus. This RNA can be translated, resulting in background levels of heterologous polypeptide. For example, in host cells comprising a recombinant inducible promoter, the molar concentration ratio of a heterologous polypeptide in an induced host cell compared to an uninduced host cell, can be, for example, about 10:1. Expression of a transgene due to leaky transcription from an inducible promoter can lead to the

same kinds of problems encountered when a constitutively active promoter is used. Therefore, it would be useful to provide a system for transgene expression in which the level of expression of the transgene in an unstimulated cell is not measurably greater than in a non-transgenic cell of the same type.

[0003] RNA viruses, nucleic acids thereof, and DNA copies of RNA viral sequences have been used to control expression of transgenes. RNA viruses can comprise single-stranded RNA or double-stranded RNA. Single-stranded RNA viruses are either "positive-strand" or "negative-strand" RNA viruses. A positive-strand single-stranded RNA virus comprises sequence in the same reading sense as viral mRNA(s). With the exception of positive strand single-stranded RNA retroviruses, which use a DNA intermediate, replication and transcription of a positive-strand RNA virus involves synthesis of a complementary (negative strand) RNA copy of the viral genome. Synthesis of a complementary RNA copy requires an RNA-dependent RNA polymerase (RDRP) encoded within the viral genome. Viral replication in a cell involves a replication complex comprising the RDRP. During the replication process, the replication complex binds to the 3' untranslated region (3' UTR) of the viral RNA, and initiates synthesis of the complementary strand (van Rossum, C.M.A., et al., *J. Gen. Virol.* 78: 3045-3049, 1997).

[0004] U.S. Patent 6,433,248 B1 to Lommel et al. discloses a method of activating transcription of an RNA of interest in a cell. The disclosed method includes the steps of (a) providing a host cell containing a heterologous construct, the heterologous construct comprising an RNA virus subgenomic promoter operatively associated with a heterologous RNA of interest, wherein the promoter does not initiate transcription of the heterologous RNA in the absence of a corresponding RNA virus trans-activating RNA segment, and where the RNA virus trans-activating RNA segment is absent from the host cell; and (b) introducing a trans-activating

nucleic acid segment into the host cell so that transcription of the heterologous RNA is initiated. The method relies on the use of a viral trans-activating RNA segment, and because of the presence of RNA comprising coding sequence for the heterologous polypeptide in an uninduced host, is still subject to background levels of expression. Furthermore, unlike the present disclosure, the method does not utilize an RNA sequence complementary to an internal ribosome entry site.

[0005] U.S. Patent 6,462,255 B1 to Thurpen discloses high level expression of foreign genes in plants using viral replicons, wherein the replicons code for at least one foreign gene and possess sequences required in *cis* for replication. Unlike the present disclosure, because of the presence of RNA comprising coding sequence for the heterologous polypeptide in an uninduced host, the methods described are still subject to background levels of expression. Furthermore, the patent does not disclose an RNA comprising an antisense coding sequence, and anti-IRES, and a 3' UTR as set forth in the present disclosure.

[0006] US Patent 6,326,480 B1 to Kovelman et al. discloses a reporter system for assaying positive strand RNA virus replication. The invention describes antisense reporter plasmids comprising a promoter operably linked to a DNA sequence encoding: (a) a sequence complementary to the 3' end of a viral genome; (b) a reporter gene in antisense orientation; and (c) a sequence complementary to the 5' end of the viral genome. The patent further describes antisense reporter mRNAs encoding: (a) a sequence complementary to a 3' end of a viral genome; (b) a reporter gene in antisense orientation; and (c) a sequence complementary to a 5' end of the viral genome. Unlike the present disclosure, this patent does not disclose a recombinant RNA comprising a sequence complementary to the coding sequence of a heterologous polypeptide, a sequence complementary to an internal ribosome entry site, and a

viral 3' untranslated region, nor does it describe a recombinant mRNA molecule comprising an internal ribosome binding site operably linked to an RNA sequence encoding a heterologous polypeptide and the complement of an internal ribosome entry site.

[0007] US Patent Application Publication US 2002/0138873 A1 to Lewandowski et al. discloses a multiple component RNA vector system, consisting of an RNA replicon comprising a 5' non-translated region, an open reading frame (ORF) homologous to an ORF of an intact or fragments of a non-structural protein of an RNA virus, a sequence non-native to the RNA virus, and a 3' non-translated region. The recombinant RNA molecules of the present invention do not require an open reading frame (ORF) homologous to an ORF of an intact or fragments of a non-structural protein of an RNA virus or a 5' non-translated region.

[0008] Powell et al. (*Proc. Natl. Acad. Sci. USA* 86: 6949-6952, 1989) disclosed transgenic tobacco plants that express RNA sequences complementary to the tobacco mosaic virus coat protein coding sequence comprising a tRNA-like structure at the 3' end of the TMV RNA. Transgenic plants that expressed RNA sequences complementary to the coat protein coding region and the 3' untranslated region, including the tRNA-like sequences, when challenged with TMV, were protected from infection at low levels of inoculum. These findings did not disclose synthesis or expression of an RNA comprising the complement of a 3' UTR, an IRES and coding sequence of a heterologous polypeptide.

[0009] Zaccomer et al (Gene 136: 87-94, 1993) reported experiments with transgenic rapeseed (*Brassica napus*) in which the transgenes comprised either a sense or antisense coding sequence of a chloramphenicol acetyltransferase (CAT) gene upstream from a positive strand 3'-terminal 100 nucleotides of the noncoding region of the turnip yellow mosaic virus. RNA complementary to the initial transcript was detected after infection of a host transgenic plant with

turnip yellow mosaic virus. These findings did not disclose synthesis or expression of an RNA comprising the complement of a 3' UTR, an IRES and coding sequence of a heterologous polypeptide.

[0010] Teycheney et al (*J. Gen. Virol.* 81: 1121-1126, 2000) reported that transcripts of transgenes comprising the 3' UTR of Lettuce mosaic virus could serve as template for synthesis of complementary negative strand RNA following infection of host tobacco plants with Tobacco etch virus, Tobacco vein mottle virus or Pepper mottle virus, but not with Cucumber mosaic virus. These workers also showed that deletion of the 3' UTR from the transgene abolished the synthesis of negative strands. These findings did not disclose synthesis or expression of an RNA comprising the complement of a 3' UTR, an IRES and coding sequence of a heterologous polypeptide.

[0011] Therefore, provision of a recombinant RNA comprising a sequence complementary to the coding sequence of a heterologous polypeptide (an "anti-sense coding sequence"), a sequence complementary to an internal ribosome entry site (an "anti-IRES"), and a viral 3' untranslated region (3' UTR) as set forth herein, and which is not expected to provide sense strand coding sequence for a heterologous polypeptide prior to stimulation or activation of synthesis of a complementary strand of the recombinant RNA, has not been reported or suggested heretofore.

SUMMARY

[0012] Accordingly, the inventors herein have succeeded in developing a transgene expression system. The system includes the provision of a recombinant RNA comprising a complementary copy of coding sequence of a heterologous polypeptide, a complementary copy

of an internal ribosome entry site (IRES) (Strauss and Strauss, Viruses and Human Disease, Academic Press, 24-25, 2002) and a 3' untranslated region of a positive strand single-stranded RNA virus. Because coding sequence is expected to be absent in the host cell or organism prior to introduction or application of a stimulus that introduces an RNA-directed RNA polymerase (RDRP) for formation of a complementary copy of the RNA in the host cell or organism, the amount of the complementary copy of the recombinant RNA and the heterologous polypeptide are expected to be no greater than that of a non-transgenic control cell or organism. Therefore, the expression levels of the RNA complement of the recombinant RNA or heterologous polypeptide in the absence of the stimulus or activating signal are expected to be below detection limits, even when highly sensitive detection means such as reverse transcription-polymerase chain reaction (for the RNA) or radioimmunoassay (for the polypeptide) are used. The amount of the RNA complement of the recombinant RNA comprised by a cell not provided a stimulus is expected to be, for example, less than about 1000 copies per cell, less than about 100 copies per cell, less than about 10 copies per cell, or zero copies per cell. Furthermore, the amount of the heterologous polypeptide comprised by a cell not provided a stimulus is expected to be, for example, less than about 1000 copies per cell, less than about 100 copies per cell, less than about 10 copies per cell, or zero copies per cell. The molar concentration ratio of RNA complementary to the recombinant RNA in a cell provided a stimulus relative to a cell not provided a stimulus is expected to be, for example, at least about 50:1, at least about 100:1, at least about 1000:1, or at least about 10,000:1. The molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is expected to be, for example, at least about 50:1, at least about 100:1, at least about 1000:1, or at least about 10,000:1.

[0013] The system can be used to express heterologous polypeptides in a host cell exposed to an activator or stimulus for synthesis of the RNA complement of the recombinant RNA. The activator or stimulus can be any extrinsic signal that results in the assembly of a replication complex in the host cell, wherein the replication complex comprises an RNA-dependent RNA polymerase. The activator or stimulus provides at least one required component of the replication complex, such as, for example, an RNA-dependent RNA polymerase. The at least one component of the replication complex can be, for example, an RNA-dependent RNA polymerase encoded by a positive strand single-stranded RNA virus. The replication complex recognizes and binds to the viral 3' UTR of the recombinant RNA, and initiates synthesis of the complementary copy of the recombinant RNA. Because the complementary copy of the recombinant RNA comprises an IRES operably linked to a sequence encoding a heterologous polypeptide, host cell ribosomes bind to and translate the sequence encoding the heterologous polypeptide.

[0014] The system can therefore be used to restrict expression to cells subjected to the activator or stimulus. The activator or stimulus can be, for example, a positive strand single-stranded RNA virus; an incomplete positive strand single-stranded RNA virus comprising the viral genes required for establishment of a replication complex, such as, for example, an RNA-dependent RNA polymerase; a virus incapable of plant-to-plant spread, or a nucleic acid thereof. The nucleic acid can be one or more RNA species comprised by a positive strand single-stranded RNA virus, or can be one or more cDNAs thereof. A vector can comprise a promoter operably linked to the cDNA.

[0015] The system can be used, for example, to express a phytotoxic polypeptide in a host cell of a plant, such as, for example, a food crop plant. Upon infection of the host cell with a

positive strand single-stranded RNA virus, such as, for example, a pathogenic plant RNA virus introduced to a plant cell by an insect or nematode vector, the phytotoxic polypeptide is expected to disable or destroy the cell, thereby protecting the plant from viral replication and spread of a disease mediated by the RNA virus. Because expression is restricted to a host cell or cells activated by a stimulus, such as, for example, a virus infection, a viral RNA tranfection or a viral cDNA transfection, the amount of the phytotoxic polypeptide can be extremely low as a percentage of total polypeptide of the host plant. Therefore, the presence of the transgene in a food crop plant or plant cell can be acceptable in terms of environmental impact, safety, or regulations concerning genetically modified foods. The system can also be used, for example, in a plant to produce a useful polypeptide, such as, for example, an economically useful polypeptide such as, for example, a pharmaceutically useful polypeptide. Because the background levels of expression in a transgenic cell or plant of either the RNA coding the polypeptide, or the polypeptide itself, are expected to be no greater than that of non-transgenic control cell or plant, and can be zero or below detection limits, it is expected that the invention will allow a plant comprising a transgene of the invention to be planted, grown or harvested with low risk of uncontrolled introduction of a heterologous polypeptide into the environment. Similarly, system can also be used, for example, in a plant to produce a polypeptide conferring disease resistance to the plant, such as, for example, a viral polypeptide such as, for example, a viral coat protein polypeptide (Fitchen and Beachy, *Annual Rev. Microbiol.* 47: 739-763, 1993; Powell-Abel et al., 1986; Powell et al., *Proc. Natl. Acad. Sci. USA* 86: 6949-6952, 1989; Zaccomer et al., *Gene* 136: 87-94, 1993).

[0016] The present invention is, therefore, directed in general to a recombinant RNA molecule comprising, in 5' to 3' direction, an anti-sense coding sequence of a heterologous

polypeptide (including an antisense translation initiation codon), a complement of an internal ribosome entry site (IRES), and a 3' untranslated region (3' UTR) of a positive strand single-stranded RNA virus, (figure 1B). Preferably, the positive strand single-stranded RNA virus is a positive strand single-stranded RNA virus having no DNA stage. A host cell can comprise the recombinant RNA sequence. The host cell is expected to synthesize neither an RNA encoding the heterologous polypeptide, nor the heterologous polypeptide itself in the absence of an activator or stimulus for synthesis of the complementary strand of the recombinant RNA. Preferably, the activator is a helper RNA virus or a portion thereof that encodes an RNA-directed RNA polymerase, or the nucleic acid thereof. Preferably, the helper virus is a positive strand single-stranded RNA virus. Preferably, the positive strand single-stranded RNA virus is a positive strand single-stranded RNA virus having no DNA stage. Preferably, the helper virus is a plant virus, such as, for example, a plant virus incapable of plant-to-plant spread. Preferably, the helper virus is a complete virus. Upon infection or transfection of a transgenic host cell expressing the recombinant RNA with a positive strand single-stranded RNA virus or the nucleic acid thereof, a viral RNA-dependent RNA polymerase is expressed, and a replication complex forms which comprises the RDRP. The replication complex binds to and initiates synthesis from the 3' UTR of the recombinant RNA. The trans-acting activator thereby stimulates synthesis of the RNA complement of the recombinant RNA in the infected cell. The RNA complement of the recombinant RNA comprises, in 5' to 3' direction, the complement of the 3' UTR, an IRES, and coding sequence for the heterologous polypeptide, wherein the IRES is operably linked to the coding sequence for the heterologous polypeptide (figure 1C). The provision of an IRES operatively linked to coding sequence of a heterologous polypeptide thereby provides an RNA molecule that a ribosome can bind (at the IRES) and translate (starting at an initiation codon of

the coding sequence) (figure 1b, figure 5b, figure 6b). Therefore, the RNA complementary to the recombinant RNA (figure 1c, figure 5c, figure 6c) is expected to bind to a ribosome at the IRES, and the coding sequence is expected to be read by the ribosome for synthesis of the heterologous polypeptide. An uninduced cell comprising the transgene does not synthesize a sense copy of the RNA sequence encoding a heterologous polypeptide, so that heterologous polypeptide synthesis is expected to be no greater than that of a control, non-transgenic cell. Therefore, the level of expression of the heterologous polypeptide in an uninduced transgenic cell or organism is expected to be, for example, less than about 1000 copies per cell, less than about 100 copies per cell, less than about 10 copies per cell, or zero copies per cell.

[0017] Thus, in one embodiment, the invention is directed to a recombinant DNA transgene encoding a recombinant RNA molecule. In preferred embodiments, the DNA transgene comprises a promoter recognized by a DNA-dependent RNA polymerase comprised by the host cell, wherein the promoter is operably linked, in the 5' to 3' direction, to a DNA sequence comprising an anti-sense coding sequence for a heterologous polypeptide, a sequence complementary to an IRES, and a 3' UTR of a positive strand single-stranded RNA virus. The 3' UTR of a positive strand single-stranded RNA virus can be a 3' UTR of a positive strand single-stranded RNA virus having no DNA stage. Preferably, the 3' UTR of a positive strand single-stranded RNA virus having no DNA stage is a 3' UTR of a positive strand single-stranded RNA plant virus having no DNA stage (figure 1a, figure 5a, figure 6a).

[0018] One embodiment of the method is the provision of a method of conferring disease resistance to a transgenic plant. The method comprises providing a transgenic plant comprising a recombinant DNA molecule comprising a promoter operably linked to a DNA sequence comprising, in the 5' to 3' direction, a sequence complementary to a coding sequence for a

heterologous polypeptide capable of conferring disease resistance, a sequence complementary to an internal ribosome entry site, a 3' UTR of a first positive strand single-stranded RNA virus; and growing the transgenic plant, whereby resistance is conferred to infection from a second positive strand single-stranded RNA virus.

[0019] The promoter can be a constitutive promoter or an inducible promoter. Preferably, the promoter is a constitutive promoter. Preferably, the promoter is a Cauliflower mosaic virus 35S promoter. In some embodiments, the DNA transgene can further comprise a cis-acting transcription terminator situated 3' to the 3' UTR.

[0020] In another aspect, the invention is directed to a host cell comprising the recombinant DNA transgene. A host cell chromosome, or an extrachromosomal a vector, such as a plasmid or virus, can comprise the DNA transgene. In preferred embodiments, the host cell is a plant cell.

[0021] In another aspect, the invention is directed to a host organism comprising the host cell comprising the recombinant DNA transgene. Preferably, the host organism is a plant, more preferably a *Nicotiana* plant, more preferably a *Nicotiana benthamiana* plant.

[0022] In another embodiment, the invention is directed to a recombinant RNA molecule, wherein the recombinant RNA molecule comprises, in 5' to 3' direction, an anti-sense coding sequence for a heterologous polypeptide, an anti-sense IRES, and a 3' UTR of a positive strand single-stranded RNA virus.

[0023] In another aspect, the invention is directed to a host cell comprising the recombinant RNA molecule. In preferred embodiments, the host cell is a plant cell.

[0024] In another aspect, the invention is directed to a host organism comprising the host cell comprising the recombinant RNA molecule. Preferably, the host organism is a plant, more preferably a *Nicotiana* plant, more preferably a *Nicotiana benthamiana* plant.

[0025] In one embodiment, the invention is directed to the complement of a recombinant RNA molecule, wherein the RNA complement of the recombinant RNA molecule comprises, in 5' to 3' direction, the complement of a 3' UTR of a positive strand single-stranded RNA virus, an IRES, and coding sequence for a heterologous polypeptide.

[0026] In another aspect, the invention is directed to a host cell comprising the RNA complement of the recombinant RNA molecule. In preferred embodiments, the host cell is a plant cell. Because the RNA complement of the recombinant RNA molecule comprises an IRES operably linked to a sequence encoding a heterologous polypeptide, the host cell is expected to express the heterologous polypeptide.

[0027] In another aspect, the invention is directed to a host organism comprising the host cell comprising the RNA complement of the recombinant RNA molecule. Preferably, the host organism is a plant, more preferably a *Nicotiana* plant, more preferably a *Nicotiana benthamiana* plant.

[0028] In another embodiment, the invention is directed to a host cell comprising a heterologous polypeptide encoded by coding sequence comprised by the complement of a recombinant RNA, wherein the RNA complement of the recombinant RNA comprises, in 5' to 3' direction, the complement of a 3' UTR of a positive strand single-stranded RNA virus, an IRES, and coding sequence for a heterologous polypeptide.

[0029] In certain aspects, the RNA virus source of the 3' UTR of the recombinant RNA molecules described herein, is preferably a positive strand single-stranded RNA virus.

Preferably, the positive strand single-stranded RNA virus is a positive strand single-stranded RNA virus having no DNA stage. A positive strand single-stranded RNA virus having no DNA stage providing a source of a 3' UTR can be a positive strand single-stranded RNA virus that infects animal cells or human cells (an "animal virus") or a positive-strand single-stranded RNA virus having no DNA stage that infects plants (a "plant virus"). Preferably, the virus is a positive strand single-stranded RNA plant virus having no DNA stage.

[0030] In certain aspects, the RNA virus that can be used to stimulate synthesis of the RNA complement of the recombinant RNA upon infection or transfection of viral nucleic acid is preferably a positive strand single-stranded RNA viruses. A positive strand single-stranded RNA virus can be a positive strand single-stranded RNA virus that infects animal cells or human cells (an "animal virus") or a positive-strand single-stranded RNA virus that infects plants (a "plant virus"). Preferably, the virus is a positive strand single-stranded RNA virus having no DNA stage. Preferably, the virus is a positive strand single-stranded RNA plant virus. Preferably, the virus produces an RNA-dependent RNA polymerase which can comprise a replication complex that can bind to the 3' UTR of the recombinant RNA and catalyze synthesis of the complement of a recombinant RNA comprising the 3' UTR. The virus used to stimulate synthesis of the RNA complement of the recombinant RNA upon infection or transfection of viral nucleic acid can be identical to the virus used as the source of the 3' UTR of the transgene. The virus used to stimulate synthesis of the RNA complement of the recombinant RNA can also be different from the virus source of the 3' UTR, provided that the replication complex formed upon infection or transfection of the stimulating viral nucleic acid recognizes the 3' UTR of the recombinant RNA. Recognition of the 3' UTR of the recombinant RNA by an infecting or transfecting virus can be

determined by standard methods known in the art (for example, the methods disclosed in Teycheney et al., *J. Gen. Virol.* 81: 1121-1126, 2000).

[0031] In another embodiment, the invention is directed to a method of synthesizing a heterologous polypeptide. The method comprises providing a transgenic host cell comprising a recombinant DNA transgene in which the cell transcribes the recombinant DNA transgene and thereby accumulates a recombinant RNA molecule, and stimulating or activating the synthesis of an RNA complementary to the recombinant RNA molecule. In this method, the recombinant DNA transgene can comprise a promoter operably linked, in 5' to 3' order, to a DNA sequence comprising a sequence complementary to the coding sequence for a heterologous polypeptide, a DNA sequence complementary to an IRES, and a DNA sequence corresponding to a 3' UTR of a positive strand single-stranded RNA virus. The transgene can also include sequence complementary to one or more intervening sequences ("introns"), and, at the 3' end, a transcription terminator. A recombinant RNA transcribed from DNA of the transgenic host cell can comprise, in 5' to 3' order, an RNA sequence complementary to the coding sequence for a heterologous polypeptide, an RNA sequence complementary to an IRES, and a 3' UTR of a positive strand single-stranded RNA virus. Stimulating or activating synthesis of an RNA complementary to the recombinant RNA can result in synthesis of an RNA sequence comprising the complement of a 3' UTR of a positive strand single-stranded RNA virus, an IRES, and coding sequence of a heterologous polypeptide, wherein the IRES and the coding sequence are operably linked. Host cell ribosomes are expected to bind to the RNA complementary to the recombinant RNA and translate the coding sequence, thereby forming the heterologous polypeptide. Stimulating the synthesis of the RNA complement of the recombinant RNA molecule can comprise infecting the host cell with a positive strand single-stranded RNA virus,

transfecting the host cell with a cDNA of a positive strand single-stranded RNA virus or transfecting the host cell with RNA of a positive strand single-stranded RNA virus. The transfecting can be by any transfection method known in the art. It is believed that RNA of a positive strand single-stranded RNA virus, upon infection or transfection of the host cell, is translated by host cell ribosomes, thereby providing polypeptide components comprised by a replication complex, such as, for example, an RNA-dependent RNA polymerase. A replication complex is expected to bind to the 3' UTR of the recombinant RNA, and initiate synthesis of an RNA complementary to the recombinant RNA starting at the 3' UTR. Elongation synthesis of RNA complementary to the recombinant RNA is expected to follow initial binding of the replication complex to the 3' UTR. Translation of the coding sequence comprised by the RNA complementary to the recombinant RNA comprises ribosomes recognizing and binding the IRES, and initiating translation of the coding sequence operably linked to the IRES. Translation of the coding sequence yields the heterologous polypeptide.

[0032] In another aspect, the invention is directed to transgenic seed comprising a recombinant DNA transgene encoding a recombinant RNA molecule. In preferred embodiments, the DNA transgene comprises a promoter recognized by a DNA-dependent RNA polymerase, wherein the promoter is operably linked, in 5' to 3' direction, to a sequence comprising an anti-sense coding sequence for a heterologous polypeptide, a sequence complementary to an IRES, and a 3' UTR of a positive strand single-stranded RNA virus. In some embodiments, the DNA transgene further comprises a cis-acting transcription terminator situated 3' to the 3' UTR. Preferably, the transgene can be integrated into the seed genome and can be present in cells of plants grown from the seed.

[0033] In another embodiment, the invention is directed to a method of making a transgenic cell comprising a recombinant DNA transgene encoding a recombinant RNA molecule. In preferred embodiments, the DNA transgene comprises a promoter recognized by a DNA-dependent RNA polymerase, wherein the promoter is operably linked, in 5' to 3' direction, to a DNA sequence comprising an anti-sense coding sequence for a heterologous polypeptide, a sequence complementary to an IRES, and a 3' UTR of a positive strand single-stranded RNA virus. The method comprises introducing the transgene to a cell. The introducing the transgene can comprise using any method known in the art to introduce heterologous DNA to a cell. For example, the introducing the recombinant DNA can comprise bombarding a cell with the DNA using a "gene gun," contacting the cell with a virus vector comprising the recombinant DNA, or contacting the cell with bacteria such as a transgenic *Agrobacterium tumefaciens* comprising a recombinant Ti plasmid comprising a transgene.

[0034] In another embodiment, the invention is directed to a DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell, the DNA molecule comprising a promoter operably linked, in the 5' to 3' direction, to at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation, an anti-IRES, and a 3' UTR of a positive strand single-stranded RNA virus. The at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation can comprise at least one recombination site and/or at least one restriction site. The at least one recombination site can be, for example, a bacteriophage lambda *att* site or a topoisomerase I-based recombination site, and the at least one restriction site can be, for example, a polylinker. The DNA molecule of this embodiment facilitates the construction of a DNA comprising, in the 5' to 3' direction, a promoter, an anti-sense coding

sequence of a heterologous polypeptide in an antisense orientation, an anti-IRES, and a 3' UTR of a positive strand single-stranded RNA virus. This DNA molecule can itself be a vector, such as, for example, a virus or a plasmid. The DNA molecule can further comprise sequences additional sequences, such as, in non-limiting example, a sequence complementary to a sequence encoding a leader peptide or a transcription termination site.

[0035] In a related aspect, the invention is directed to a method of making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell. The method comprises providing a DNA molecule comprising a promoter operably linked, in the 5' to 3' direction, to at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation, a sequence complementary to an internal ribosome entry site, and a 3' UTR of a positive strand single-stranded RNA virus, and inserting a sequence encoding a heterologous polypeptide into the insertion site of the DNA molecule in an antisense orientation relative to the direction of transcription from the promoter. The inserting can be by any means known in the art. A recombinant DNA molecule comprising a promoter operably linked to a DNA sequence comprising, in the 5' to 3' direction, a sequence complementary to a coding sequence for a heterologous polypeptide, a sequence complementary to an internal ribosome entry site and a 3' UTR of a positive strand single-stranded RNA virus.

[0036] In a related embodiment, the invention is directed to a kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell. The kit can comprise a DNA molecule comprising a promoter operably linked, in the 5' to 3' direction, to at least one site for incorporation of coding sequence of a heterologous polypeptide in an antisense orientation, an anti-IRES, and a 3' UTR of a positive strand single-stranded RNA virus, and packaging. A user of the kit can, for example, incorporate coding sequence for a heterologous polypeptide into the

DNA vector such that transcription of the vector would yield a transcript comprising, in the 5' to 3' direction, the complement of the coding sequence, the complement of the IRES, and the 3' UTR. In some aspects, the kit can further comprise a positive strand single-stranded RNA virus or nucleic acid thereof that, upon infection or transfection, would support the formation of an RNA complementary to the recombinant RNA. In some aspects, the kit can further comprise a host organism for growing the vector, such as, for example, transformation-competent *E. coli*. In some aspects, the kit can further comprise instructions.

[0037] In a related aspect, the invention is directed to a method of forming a host cell comprising a transgene comprising a promoter operatively linked to a DNA sequence comprising, in 5' to 3' order, a sequence complementary to a sequence encoding a heterologous polypeptide, a sequence complementary to an IRES, and a sequence comprising a 3' UTR of an RNA virus. The method comprises transforming or infecting the host cell with the DNA. The transforming or infecting can be by any method known in the art.

BRIEF DESCRIPTION OF THE DRAWINGS

[0038] Figure 1 illustrates the organization of nucleic acids described herein. (a) A DNA transgene, wherein "Promoter" represents a promoter for transcription of the transgene; "α-coding" represents DNA sequence complementary to coding sequence of a heterologous polypeptide; "α-IRES" represents DNA sequence complementary to DNA sequence encoding an IRES; and "3' UTR" represents DNA sequence corresponding to a 3' untranslated region of a positive strand single-stranded RNA virus. Polarity of the DNA is indicated by "5'" and "3'" at the ends, and by an arrow at the 3' end. (b) A recombinant RNA, wherein "α-coding" represents anti-sense coding sequence of a heterologous polypeptide; "α-IRES" represents RNA sequence complementary to an IRES; and "3' UTR" represents RNA sequence of a 3' untranslated region

of a positive strand single-stranded RNA virus. Polarity of the RNA is indicated by "5'" and "3'" at the ends, and by an arrow at the 3' end. (c) An RNA complementary to the recombinant RNA of (b), wherein "α-3' UTR" represents RNA sequence complementary to a 3' UTR of a positive strand single-stranded RNA virus; "IRES" represents an internal ribosome entry site, and "coding" represents RNA sequence encoding a heterologous polypeptide.

[0039] Figure 2 illustrates plasmids used to demonstrate synthesis of RNA complementary to a recombinant RNA upon host cell infection with a positive strand single-stranded RNA virus. Wild type Cowpea chlorotic mosaic virus RNA3 has a 3a gene open reading frame (ORF) and a coat protein ORF, and is maintained in plasmid pCC3TP4. A *Not I* restriction site was introduced near the 3' end of the coat protein gene ORF in plasmid pCC3AG1 as well as the viral transgenes in transgenic plants 3-57 and Δ69 (Greene and Allison, *Science* 263: 1423-1425, 1994; Greene and Allison, *Virology* 225: 231-234, 1996). Transgenic plant 3-57 was transformed with the 3' 2/3 of the coat protein ORF and the full-length 3' UTR. Transgenic plant Δ69 was transformed with the same viral gene, but the 3' UTR bears a 69-nucleotide deletion at the 3' end. The negative sense RNA-specific primer RA83 (5'-AAGTGGATCCCCTC TTGTGCGGCTGC-3' (SEQ ID NO: 1)) anneals at nucleotides 1519-1544, and was used for first strand cDNA synthesis and for PCR. An additional primer RA84 (5'-ACTCCAAAGAGTTCTTCCG-3' (SEQ ID NO: 2)) anneals at nucleotides 2072-2090, and was used for PCR.

[0040] Figure 3 illustrates agarose gel electrophoresis showing RT-PCR-amplified negative-sense Cowpea chlorotic mottle virus (CCMV) RNA3. Total RNA from virus infected or mock-inoculated transgenic and non-transgenic *N. benthamiana* plant tissue was treated with RNase-free DNase I to remove genomic DNA. A negative-sense CCMV RNA3-specific primer

RA83 (5'-AAGTGGATCCCCTC TTGTGCGGCTGC-3' (SEQ ID NO: 1)), which anneals at nucleotides 1519-1544, was used for first-strand cDNA synthesis. RA83 and an additional primer RA84 (5'-ACTCCAAAGAGTTCTTCCG-3' (SEQ ID NO: 2)), which anneals at nucleotides 2072-2090, were used for PCR amplification. Lanes 5-7, 8-10, and 11-13 were from nontransgenic, transgenic Δ69, and 3-57 plants, respectively. Samples in lanes 5, 8 and 11 were mock-inoculated (M); lanes 6, 9 and 12 were inoculated with Brome mosaic virus (B): lanes 7, 10 and 13 were inoculated with CCMV (C). Lane 1 contains PCR product using 0.1 μg pCC3AG1 plasmid DNA. Lane 2 is a negative control of PCR in which water was added to the PCR mixture. Lane 3 is empty (E). Lane 4 comprises 1 kb size markers (Gibco BRL).

[0041] Figure 4 illustrates an agarose gel showing *Not I*-digested RT-PCR products amplified from total RNA extracted from virus infected plant tissue. Lane 1 comprises a 1 kb size marker (Gibco BRL). Lanes 2-3 comprise PCR products amplified from pCC3AG1. Lanes 4-5 comprise RT-PCR-amplified products from CCMV-infected non-transgenic *N. benthamiana* plants. Lanes 6-7 comprise RT-PCR-amplified products from CCMV-infected Δ69 plants. Lanes 8-9 comprise RT-PCR-amplified products from BMV infected 3-57 plants. Lanes 10-11 comprise RT-PCR-amplified products from CCMV-infected 3-57 plants. RT-PCR products in lanes 2, 4, 6, 8 and 10 were not treated with *Not I* restriction enzyme. RT-PCR products in lanes 3, 5, 7, 9 and 11 were digested with *Not I* restriction enzyme. An arrow "←" indicates undigested fragments. An arrowhead "◀" indicates *Not I* –digested fragments (in lanes 3, 9 and 11; note small digested bands in lane 11).

[0042] Figure 5 illustrates generalized arrangement of components *in planta*. (a) Double strand DNA transgene complex. The plant RNA polymerase II recognizes the transcriptional promoter and produces the RNA transcript shown in (b). (b) An RNA polymerase II (Pol II)

transcript of the DNA transgene shown in (a). The Pol II transcript is transported to the cytoplasm where it awaits the virus that recognizes its 3' UTR as a replication initiation site. Upon introduction of the appropriate RNA virus, the viral replication complex recognizes its 3' UTR and makes a complementary RNA copy (c) of the Pol II transcript. (c) A complementary RNA copy of the Pol II transcript shown in (b). The functional IRES enables the entry of a ribosome and the translation of the transgene that is now in the sense orientation.

[0043] Figure 6 illustrates a detailed arrangement of components *in planta*. (a) illustrates the antisense relationship of the gene and IRES with respect to the promoter and the viral 3' UTR. Upside down nucleotides indicate antisense orientation. (b) illustrates the RNA polymerase transcript with the gene and IRES in upside down antisense orientation. (c) illustrates final conversion resulting in both the IRES and gene in a translatable orientation. AATTCC indicates IRES; ATG indicates initiation codon; XXX indicates any codon; YYY indicates complements of a codon; asterisk indicates a stop codon.

DETAILED DESCRIPTION

[0044] Molecular biology handbooks, such as Sambrook, J., et al., Molecular Cloning: A Laboratory Manual; Cold Spring Harbor Laboratory: Plainview, NY (1990) provide guidance for standard molecular biology methods used herein. Where examples are recited herein, such examples are intended to be non-limiting.

[0045] In one embodiment, the invention is directed to a recombinant DNA transgene encoding a recombinant RNA molecule. In preferred embodiments, the DNA transgene comprises a promoter recognized by a DNA-dependent RNA polymerase, wherein the promoter is operably linked, in 5' to 3' direction, to a DNA sequence comprising an anti-sense coding

sequence for a heterologous polypeptide, a sequence complementary to an IRES, and a 3' UTR of a positive strand single-stranded RNA virus.

[0046] The recombinant DNA transgene comprising the recombinant mRNA can further comprise a vector. The vector can be a plasmid, phagemid, or virus. The plasmid can be any plasmid suitable for use as a vector, in non-limiting example, a pBR322, a pBluescript® plasmid (Stratagene, La Jolla, CA), or a pUC plasmid. The virus is any virus suitable for use as a vector, such as, for example, a bacteriophage, such as, for example, a lambda bacteriophage. The vector can comprise sequences, such as, in non-limiting example, a prokaryotic origin of replication, a eukaryotic origin of replication, one or more selectable markers, such as, for example, a gene encoding a polypeptide that provides antibiotic resistance, such as, for example, a beta-lactamase, a polylinker, and one or more prokaryotic promoters such as, for example, a bacteriophage T3 promoter, a bacteriophage T7 promoter, or a bacteriophage Sp6 promoter. A host organism for the bacteriophage or plasmid can be any suitable prokaryotic host organism, such as, for example, an *E. coli*. The virus can also be a DNA virus or an RNA virus that can be comprised by a eukaryotic cell, such as an animal cell, a plant cell, or a cell of a microorganism such as, for example, yeast.

[0047] The promoter can be a eukaryotic promoter, and can be a eukaryotic constitutive promoter or a eukaryotic inducible promoter. The promoter can be, for example, a promoter known in the art (e.g., Praz et al., Nucleic Acids Research, 30: 322-324, 2002). The promoter can be, for example, a promoter of a naturally-occurring gene, a synthetic promoter, or a promoter of a naturally occurring gene which has been modified to alter transcription levels and/or tissue specificity. Preferably, the promoter is a eukaryotic constitutive promoter, such as, for example, a cauliflower mosaic virus (CaMV) 35S promoter, a blueberry red ringspot virus promoter, a

ubiquitin gene promoter such as, for example, a maize ubiquitin 1 promoter (Cornejo et al., *Plant Mol. Biol.* 23: 567-581, 1993), an actin gene promoter such as, for example, a β-actin promoter or a rice actin-1 gene promoter (McElroy et al., *Plant Cell* 2: 163-71, 1990), an NeIF-4A10 promoter (Mandel et al., *Plant Mol. Biol.* 29: 995-1004, 1995), a maize Adh1-based pEmu promoter, (Wilmink et al., *Plant Mol. Biol.* 28: 949-955, 1995), a barley leaf thionin BTH6 promoter (Holtorf et al., *Plant Mol. Biol.* 29: 637-646, 1995), a cassava vein mosaic virus (CVMV) promoter (Verdaguer et al., *Plant Mol. Biol.* 31: 1129-1139, 1996), a sugarcane bacilliform badnavirus promoter (Schenk et al., *Plant Mol. Biol.* 39: 1221-1230, 1999) or a histone gene promoter (Lepetit et al., *Mol. Gen. Genet.* 231: 276-285, 1992). Preferably, the promoter is a CaMV 35S promoter. The CaMV 35S promoter can comprises, for example, the sequence:

AGATTAGCCTTTTCAATTTCAGAAAGAATGCTAACCCACAGATGGTTAGAGAGGCTT
ACGCAGCAGGTCTCATCAAGACGATCTACCCGAGCAATAATCTCCAGGAAATCAAA
TACCTTCCCAAGAAGGTTAAAGATGCAGTCAAAAGATTCAGGACTAACTGCATCAA
GAACACAGAGAAAGATATATTTCTCAAGATCAGAAGTACTATTCCAGTATGGACGA
TTCAAGGCTTGCTTCACAAACCAAGGCAAGTAATAGAGATTGGAGTCTCTAAAAAG
GTAGTTCCCACTGAATCAAAGGCCATGGAGTCAAAGATTCAAATAGAGGACCTAAC
AGAACTCGCCGTAAAGACTGGCGAACAGTTCATACAGAGTCTCTTACGACTCAATG
ACAAGAAGAAAATCTTCGTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAA
AATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAG
GGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTG
AAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAA
GGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCA

CGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGAT
TGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAA
GACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGAACACG (SEQ ID NO: 3)

[0048] In the DNA sequence comprising an anti-sense coding sequence for a heterologous polypeptide, non-limiting examples of the heterologous polypeptide encoded by the complement of the anti-sense coding sequence, include, for illustrative purposes only, hormones and hormone precursors, such as, for example, thyroid-stimulating hormone, follicle-stimulating hormone, luteinizing hormone, prolactin, growth hormone, adrenocorticotropic hormone, growth hormone-releasing hormone, corticotropin-releasing hormone, somatostatin, calcitonin, parathyroid hormone, human chorionic gonadotropin, insulin, glucagon, somatostatin, erythropoietin, atrial-natriuretic peptide, gastrin, secretin, cholecystokinin, somatostatin, neuropeptides, insulin-like growth factor-1, angiotensinogen, thrombopoietin and leptin; enzymes, such as, for example, oxidoreductases such as, for example, dehydrogenases, oxidases, reductases and catalases; transferases such as, for example, acetyltransferases, methylases, protein kinases and phosphatases; hydrolases including proteases, nucleases and phosphatases such as, for example, alkaline phosphatase or phytase; lyases including decarboxylases and aldolases; isomerases, such as, for example, epimerases and racemases; and ligases such as, for example, peptide synthases, aminoacyl-tRNA synthetases, DNA ligases and RNA ligases; cell toxins such as, for example, barnase; cell surface proteins such as, for example, transport proteins and receptor proteins; intracellular proteins such as, for example, proteins associated with intracellular signaling such as G-proteins and associated receptors, proteins associated with intracellular transport; structural proteins; reporter proteins such as, for example, beta-galactosidase and fluorescent proteins such as a green fluorescent protein; proteins conferring

disease resistance, such as, for example, a viral coat protein polypeptide; antibodies, such as, for example, a "plantibody" (Gibbs, WW. *Scientific American* 277: 44, 1997), and numerous other proteins and polypeptides. The polypeptide can comprise, for example, a naturally occurring amino acid sequence, or conservative amino acid substitutions, deletions, or additions thereof which do not destroy the polypeptide's activity. Thus, the polypeptide can also comprise additional sequences, such as, for example, a leader sequence for cell secretion; a target sequence for a biotinylation reaction catalyzed by a biotin ligase; a polyhistidine sequence for purification on a heavy metal ion column such as, for example, a zinc ion column; an epitope tag, such as, for example, a FLAG sequence or a myc epitope tag; and a protease recognition site, such as, for example, an enterokinase recognition site. The DNA sequence comprising an anti-sense coding sequence for a heterologous polypeptide can comprise an artificial sequence or a naturally occurring DNA sequence. A DNA sequence encoding a polypeptide can encode translation codons that reflect the preferred codon usage of a host cell or organism. For example, if the host cell or organism species is *Nicotiana benthamiana*, a codon usage table such as that published on the internet at http://www.kazusa.or.jp/codon/cgi-bin/showcodon.cgi?species=Nicotiana+benthamiana+[gbpln] can be used to select codons or their complements in designing an artificial DNA sequence or modifying a naturally occurring DNA sequence. It is expected that use of preferred codons in a coding sequence will lead to higher efficiency of translation of a transgene in a transgenic cell or organism. The DNA sequence comprising an antisense coding sequence for a heterologous polypeptide can further comprise one or more antisense introns, at least one antisense translation termination codon, and a transcription termination signal.

[0049] The DNA sequence complementary to an IRES can comprise a sequence complementary to any known IRES. The IRES can be, for example, any IRES known in the art to function to support internal ribosomal entry of an RNA in a eukaryotic cell. The IRES, therefore, may derive from any number of different viruses, animals, plants, or eukaryotic microorganisms, or may be an artificial IRES. Non-limiting examples of an IRES that can be used in the invention include those retrievable from an internet database (Bonnal et al., *Nucleic Acids Res.* 31: 427-428, 2003). Non-limiting examples of an IRES include a picornavirus IRES (Jang et al, *Enzyme* 44: 292-309, 1990; Roberts et al., *RNA* 4: 520-529, 1998), a foot-and-mouth disease virus IRES (Kuhn et al., *J. Virol.* 64: 4625-4631, 1990); an encephalomyocarditis virus IRES (Evstafieva et al., *Nucleic Acids Res.* 19: 665-671, 1991), a hepatitis A virus IRES (Brown et al., *J. Virol.* 65: 5828-5838, 1991), a hepatitis C virus IRES (Tsukiyama-Kohara et al., *J. Virol.* 66: 1476-1483, 1992), a human rhinovirus IRES (Borman et al., *Virology* 188: 685-696, 1992), a poliovirus IRES (Haller et al, *J. Virol.* 66: 5075-5086, 1992; Klinck et al., *Nucleic Acids Res.* 25: 2129-2137, 1997), a swine vesicular disease virus IRES (Chen et al., *J. Virol.* 67: 2142-2148, 1993), a turnip mosaic potyvirus IRES (Basso et al., *J. Gen. Virol.* 75: 3157-3165, 1994), a human fibroblast growth factor 2 mRNA IRES (Vagner et al., *Mol. Cell Biol.* 15: 35-44, 1995), a pestivirus IRES (Poole et al., *Virology* 206: 750-754, 1995), a Leishmania RNA virus IRES (Maga et al., *Mol. Cell Biol.* 15: 4884-4889, 1995), a Moloney murine leukemia virus IRES (Vagner S, *J. Biol. Chem.* 270: 20376-20383, 1995), a human rhinovirus 14 IRES (Rojas-Eisenring et al., *J. Virol.* 1995 69: 6819-6824, 1995), an aphthovirus IRES (Martinez-Salas et al., *J. Virol.* 70: 992-998, 1996), a human immunoglobulin heavy chain binding protein (BiP) mRNA IRES (Le et al., *Nucleic Acids Res.* 25: 362-369, 1997), a *Drosophila* Antennapedia mRNA IRES (Le et al., *Nucleic Acids Res.* 25: 362-369, 1997), a human fibroblast growth factor

2 (FGF-2) mRNA IRES (Le et al., *Nucleic Acids Res.* 25: 362-369, 1997), a hepatitis G virus IRES (Pickering et al., *J. Viral. Hepat.* 4: 175-184, 1997), a tobamovirus IRES (Ivanov et al., *Virology* 232: 32-43, 1997), a vascular endothelial growth factor mRNA IRES (Stein et al., *Mol. Cell Biol.* 18: 3112-3119, 1998), a Coxsackie B group virus IRES (Carthy et al., Clin. Exp. Pharmacol. Physiol. 24: 997-1003, 1997), a c-myc protooncogene mRNA IRES (Nanbru et al., *J. Biol. Chem.* 272: 32061-32066, 1997; Nanbru et al., Oncogene 20:4270-4280, 2001), a human MYT2 mRNA IRES (Kim et al., *Mol. Cell* Neurosci. 12:119-140, 1998), a human parechovirus type 1 virus IRES (Ghazi et al., *J. Gen. Virol.* 79: 2641-2650, 1998), a human parechovirus type 2 virus IRES (Ghazi et al., *J. Gen. Virol.* 79: 2641-2650, 1998), a eukaryotic initiation factor 4GI mRNA IRES (Johannes et al., *RNA* 4: 1500-1513, 1998), a Plautia stali intestine virus IRES (Sasaki et al., *J. Virol.* 73: 1219-1226, 1999), a Theiler's murine encephalomyelitis virus IRES (Yamasaki et al., *J. Virol.* 73: 8519-8526, 1999), a bovine enterovirus IRES (Zell et al., *J. Gen. Virol.* 80: 2299-2309, 1999), a connexin 43 mRNA IRES (Schiavi et al., *FEBS Lett.* 464: 118-122, 1999), a homeodomain protein Gtx mRNA IRES (Chappell et al., *Proc. Natl. Acad. Sci. USA* 97: 1536-1541, 2000), an AML1 transcription factor mRNA IRES (Pozner et al., *Mol. Cell.* Biol. 20: 2297-2307, 2000), an NF-kappa B repressing factor mRNA IRES (Oumard et al., *Mol. Cell.* Biol. 20: 2755-2759, 2000), an X-linked inhibitor of apoptosis (XIAP) mRNA IRES (Holcik et al., *Mol. Cell Biol.* 20: 4648-4657, 2000), a cricket paralysis virus RNA IRES (Wilson et al., *Mol. Cell Biol.* 20: 4990-4999, 2000), a p58(PITSLRE) protein kinase mRNA IRES (Cornelis et al. *Mol. Cell* 5: 597-605, 2000), an ornithine decarboxylase mRNA IRES (Pyronnet et al., *Mol. Cell* 5: 607-616, 2000), a connexin-32 mRNA IRES (Hudder et al., *J. Biol. Chem.* 275: 34586-34591, 2000), a bovine viral diarrhea virus IRES (Sanderbrand et al., *Vet. Microbiol.* 77: 215-227, 2000), an insulin-like growth factor I receptor mRNA IRES (Giraud et al., *J. Biol.*

Chem. 276: 5668-5675, 2001), a human immunodeficiency virus type 1 gag gene IRES (Buck et al., *J. Virol.* 75: 181-191, 2001), a classical swine fever virus IRES (Kolupaeva et al., *RNA* 6: 1791-1807, 2000), a Kaposi's sarcoma-associated herpesvirus IRES (Grundhoff et al., *J. Virol.* 75: 1857-1863), a short IRES selected from libraries of random oligonucleotides (Owens et al., *Proc. Natl. Acad. Sci. USA* 98: 1471-1476, 2001), 2001; Bieleski et al., *J. Virol.* 75: 1864-1869, 2001), a Jembrana disease virus IRES (Metharom et al., *Vet. Microbiol.* 80: 9-22, 2001), an apoptotic protease-activating factor 1 mRNA IRES (Mitchell et al., *Mol. Cell Biol.* 21: 3364-3374, 2001), a Rhopalosiphum padi virus IRES (Woolaway et al., *J. Virol.* 75: 10244-10249, 2001), a cationic amino acid transporter mRNA IRES (Fernandez et al., *J. Biol. Chem.* 277: 11780-11787, 2002), a human insulin-like growth factor II leader 2 mRNA IRES (Pedersen et al., *Biochem. J.* 363: 37-44, 2002), a giardiavirus IRES (Garlapati et al., *RNA* 8: 601-611, 2002), a Smad5 mRNA IRES (Shiroki et al., *Nucleic Acids Res.* 30: 2851-2861, 2002), a porcine teschovirus-1 talfan IRES (Kaku et al., *J. Virol.* 76: 11721-11728, 2002), a *Drosophila* Hairless mRNA IRES (Maier et al., *Proc. Natl. Acad. Sci. USA* 99:15480-15485, 2002), an hSNM1 mRNA IRES (Zhang et al., *DNA Repair (Amst)* 1: 379-390, 2002), a Cbfa1/Runx2 mRNA IRES (Xiao et al., *J. Cell Biochem.* 88: 493-505, 2003), an Epstein-Barr virus IRES (Isaksson et al., Oncogene 22: 572-581, 2003), a hibiscus chlorotic ringspot virus IRES (Koh et al., *J. Biol. Chem. in press,* published on the internet at http://www.jbc.org/cgi/reprint/M210212200v1.pdf), a rat pituitary vasopressin V1b receptor mRNA IRES (Aguilera et al., *J. Mol. Endocrinol.* 30: 99-108, 2003), and a human hsp70 mRNA IRES (Rubtsova et al, J. Biol. Chem. in press, available on the internet at http://www.jbc.org/cgi/reprint/M303213200v1.pdf).

[0050] In preferred embodiments, the IRES can be the picornavirus IRES, such as, for example, the encephalomyocarditis virus IRES comprised by plasmid pIRES (BD Biosciences

Clontech, Palo Alto, CA). A DNA copy of the picornavirus internal ribosome entry site can comprise, for example, the sequence:

AATTCCGCCCCTCTCCCTCCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAAT
AAGGCCGGTGTGCGTTTGTCTATATGTGATTTTCCACCATATTGCCGTCTTTTGGCAA
TGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTCCTAGGGGTCTTTC
CCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCT
GGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACC
CCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCT
GCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGT
CAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTAC
CCCATTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTC
GAGGTTAAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCCTTTGAA
AAACACGATGATAA (SEQ ID NO: 4).

[0051] An RNA copy of the IRES can comprise, for example, the sequence:

AAUUCCGCCCCUCUCCCUCCCCCCCCCCUAACGUUACUGGCCGAAGCCGCUUGGAA
UAAGGCCGGUGUGCGUUUGUCUAUAUGUGAUUUUCCACCAUAUUGCCGUCUUUU
GGCAAUGUGAGGGCCCGGAAACCUGGCCCUGUCUUCUUGACGAGCAUUCCUAGGG
GUCUUUCCCCUCUCGCCAAAGGAAUGCAAGGUCUGUUGAAUGUCGUGAAGGAAG
CAGUUCCUCUGGAAGCUUCUUGAAGACAAACAACGUCUGUAGCGACCCUUUGCAG
GCAGCGGAACCCCCCACCUGGCGACAGGUGCCUCUGCGGCCAAAAGCCACGUGUA
UAAGAUACACCUGCAAAGGCGGCACAACCCCAGUGCCACGUUGUGAGUUGGAUAG
UUGUGGAAAGAGUCAAAUGGCUCUCCUCAAGCGUAUUCAACAAGGGGCUGAAGG
AUGCCCAGAAGGUACCCCAUUGUAUGGGAUCUGAUCUGGGGCCUCGGUGCACAUG

CUUUACAUGUGUUUAGUCGAGGUUAAAAAAACGUCUAGGCCCCCCGAACCACGGG

GACGUGGUUUUCCUUUGAAAAACACGAUGAUAA (SEQ ID NO: 5).

[0052] A DNA complementary to the IRES can comprise, for example, the sequence:

TTATCATCGTGTTTTTCAAAGGAAAACCACGTCCCCGTGGTTCGGGGGGCCTAGACG

TTTTTTTAACCTCGACTAAACACATGTAAAGCATGTGCACCGAGGCCCCAGATCAGA

TCCCATACAATGGGGTACCTTCTGGGCATCCTTCAGCCCCTTGTTGAATACGCTTGA

GGAGAGCCATTTGACTCTTTCCACAACTATCCAACTCACAACGTGGCACTGGGGTTG

TGCCGCCTTTGCAGGTGTATCTTATACACGTGGCTTTTGGCCGCAGAGGCACCTGTC

GCCAGGTGGGGGGTTCCGCTGCCTGCAAAGGGTCGCTACAGACGTTGTTTGTCTTCA

AGAAGCTTCCAGAGGAACTGCTTCCTTCACGACATTCAACAGACCTTGCATTCCTTT

GGCGAGAGGGGAAAGACCCCTAGGAATGCTCGTCAAGAAGACAGGGCCAGGTTTCC

GGGCCCTCACATTGCCAAAAGACGGCAATATGGTGGAAAATCACATATAGACAAAC

GCACACCGGCCTTATTCCAAGCGGCTTCGGCCAGTAACGTTAGGGGGGGGGGAGGG

AGAGGGGCGGAATT (SEQ ID NO: 6).

[0053] An RNA copy of the complement of the IRES can comprise, for example, the sequence:

UUAUCAUCGUGUUUUUCAAAGGAAAACCACGUCCCCGUGGUUCGGGGGGCCUAG

ACGUUUUUUUUAACCUCGACUAAACACAUGUAAAGCAUGUGCACCGAGGCCCCAGA

UCAGAUCCCAUACAAUGGGGUACCUUCUGGGCAUCCUUCAGCCCCUUGUUGAAUA

CGCUUGAGGAGAGCCAUUUGACUCUUUCCACAACUAUCCAACUCACAACGUGGCA

CUGGGGUUGUGCCGCCUUUGCAGGUGUAUCUUAUACACGUGGCUUUUGGCCGCA

GAGGCACCUGUCGCCAGGUGGGGGGUUCCGCUGCCUGCAAAGGGUCGCUACAGAC

GUUGUUUGUCUUCAAGAAGCUUCCAGAGGAACUGCUUCCUUCACGACAUUCAACA

GACCUUGCAUUCCUUUGGCGAGAGGGGAAAGACCCCUAGGAAUGCUCGUCAAGA AGACAGGGCCAGGUUUCCGGGCCCUCACAUUGCCAAAAGACGGCAAUAUGGUGGA AAAUCACAUAUAGACAAACGCACACCGGCCUUAUUCCAAGCGGCUUCGGCCAGUA ACGUUAGGGGGGGGGGAGGGAGAGGGGCGGAAUU (SEQ ID NO: 7).

[0054] The 3' UTR of the transgene can be a DNA copy of any known positive strand single-stranded RNA 3' UTR, no DNA stage. The 3' UTR sequence can be that of any known sequence of a positive strand single-stranded RNA virus, preferably a 3' UTR of a positive strand single-stranded RNA virus having no DNA stage, such as, for example a 3' UTR of a positive strand single-stranded RNA virus having no DNA stage selected from the group consisting of Aconitum latent virus, Acute bee paralysis virus, Acyrthosiphon pisum virus, Aichi virus, Alfalfa mosaic virus, Alkhurma virus, American plum line pattern virus, Aphid lethal paralysis virus, Apoi virus, Apple chlorotic leaf spot virus, Apple latent spherical virus, Apple mosaic virus, Apple stem grooving virus, Apple stem pitting virus, Artichoke mottled crinkle virus, Aura virus, Avian encephalomyelitis virus, Avian infectious bronchitis virus, Avian nephritis virus, Bacteriophage AP205, Bacteriophage M11, Bacteriophage SP, Bamboo mosaic virus, Banana mild mosaic virus, Barley mild mosaic virus, Barley stripe mosaic virus, Barley yellow dwarf virus - GAV, Barley yellow dwarf virus - MAV, Barley yellow dwarf virus - PAV, Barley yellow dwarf virus-PAS, Barley yellow mosaic virus, Barmah Forest virus, Bean common mosaic necrosis virus, Bean common mosaic virus, Bean leafroll virus, Bean pod mottle virus, Bean yellow mosaic virus, Beet black scorch virus, Beet chlorosis virus, Beet mild yellowing virus, Beet necrotic yellow vein virus, Beet ringspot virus, Beet soil-borne mosaic virus, Beet soil-borne virus, Beet virus Q, Beet western yellows ST9 associated virus, Beet western yellows virus, Beet yellows virus, Black beetle virus, Black queen cell virus, Blackcurrant reversion

virus, Blueberry scorch virus, Boolarra virus, Botrytis virus F, Bovine coronavirus, Bovine enterovirus, Bovine kobuvirus, Bovine viral diarrhea virus genotype 2, Broad bean mottle virus, Broad bean necrosis virus, Broad bean wilt virus 2, Brome mosaic virus, Brome streak mosaic virus, Cactus virus X, Calicivirus strain NB, Canine calicivirus, Cardamine chlorotic fleck virus, Carnation Italian ringspot virus, Carnation mottle virus, Carnation ringspot virus, Carrot mottle mimic virus, Cassava common mosaic virus, Cell fusing agent virus, Cereal yellow dwarf virus-RPS, Cereal yellow dwarf virus-RPV, Chayote mosaic tymovirus, Cherry green ring mottle virus, Cherry mottle leaf virus, Cherry necrotic rusty mottle virus, Cherry virus A, Chikungunya virus, Chinese wheat mosaic virus, Citrus leaf blotch virus, Citrus leaf rugose virus, Citrus tristeza virus, Clover yellow mosaic virus, Clover yellow vein virus, Cocksfoot mottle virus, Cocksfoot streak virus, Cowpea aphid-borne mosaic virus, Cowpea chlorotic mottle virus, Cowpea mosaic virus, Cowpea mottle virus, Cowpea severe mosaic virus, Cricket paralysis virus, Crucifer tobamovirus, Cryphonectria parasitica mitovirus 1-NB631, Cucumber Bulgarian virus, Cucumber fruit mottle mosaic virus, Cucumber green mottle mosaic virus, Cucumber mosaic virus, Cucumber necrosis virus, Cucumber yellows virus, Cucurbit aphid-borne yellows virus, Cucurbit yellow stunting disorder virus, Cycas necrotic stunt virus, Cymbidium mosaic virus, Cymbidium ringspot virus, Dasheen mosaic virus, Deer tick virus, Dengue virus, Drosophila C virus, Eastern equine encephalitis virus, Eggplant mosaic virus, Elm mottle virus, Encephalomyocarditis virus, Enterobacteria phage fr, Enterobacteria phage GA, Enterobacteria phage KU1, Enterobacteria phage MX1, Enterobacteria phage NL95, Enterobacterio phage MS2, Enterovirus Yanbian 96-83csf, Epinephelus tauvina nervous necrosis virus, Equine arteritis virus, Equine rhinitis A virus, Equine rhinitis B virus, Equine rhinovirus 3, Erysimum latent virus, Euprosterna elaeasa virus, European brown hare syndrome virus, Feline calicivirus, Flock

house virus, Foot-and-mouth disease virus C, Foot-and-mouth disease virus O, Foot-and-mouth disease virus SAT 2, Foxtail mosaic virus, Galinsoga mosaic virus, Garlic latent virus, Garlic virus A, Garlic virus C, Garlic virus E, Garlic virus X, Grapevine chrome mosaic virus, Grapevine fanleaf virus, Grapevine fleck virus, Grapevine leafroll-associated virus 3, Grapevine rootstock stem lesion associated virus, Grapevine virus A, Grapevine virus B, Groundnut rosette virus, Helicoverpa armigera stunt virus, Hepatitis A virus, Hepatitis C virus, Hepatitis E virus, Hepatitis G virus, Hepatitis GB virus A, Hepatitis GB virus B, Hepatitis GB virus C, Hibiscus chlorotic ringspot virus, Himetobi P virus, Hop latent virus, Human astrovirus, Human coronavirus 229E, Human echovirus 1, Human enterovirus A, Human enterovirus B, Human enterovirus C, Human enterovirus D, Human enterovirus E, Human parechovirus 2, Human rhinovirus 89, Human rhinovirus B, Igbo Ora virus, Indian citrus ringspot virus, Indian peanut clump virus, Infectious flacherie virus, Japanese encephalitis virus, Japanese iris necrotic ring virus, Japanese yam mosaic virus, Johnsongrass mosaic virus, Kashmir bee virus, Kennedya yellow mosaic virus, Kyuri green mottle mosaic virus, Lactate dehydrogenase-elevating virus, Langat virus, Leek white stripe virus, Leek yellow stripe potyvirus, Lettuce infectious yellows virus, Lettuce mosaic virus, Little cherry virus 1, Ljungan virus, Louping ill virus, Lucerne transient streak virus, Maize chlorotic dwarf virus, Maize chlorotic mottle virus, Maize dwarf mosaic virus, Maize rayado fino virus, Mayaro virus, Melon necrotic spot virus, Mink astrovirus, Modoc virus, Montana myotis leukoencephalitis virus, Murine hepatitis virus, Murray Valley encephalitis virus, Mushroom bacilliform virus, Narcissus mosaic virus, Nodamura virus, Norwalk virus, Nudaurelia capensis beta virus, O'nyong-nyong virus, Oat blue dwarf virus, Oat chlorotic stunt virus, Oat golden stripe virus, Oat mosaic virus, Obuda pepper virus, Odontoglossum ringspot virus, Olive latent virus 1, Olive latent virus 2, Ononis yellow mosaic

virus, Ophiostoma mitovirus 3a, Ophiostoma novo-ulmi mitovirus 4-Ld, Ophiostoma novo-ulmi mitovirus 5-Ld, Ophiostoma novo-ulmi mitovirus 6-Ld, Ovine astrovirus, Oyster mushroom spherical virus, Panicum mosaic virus, Papaya mosaic virus, Papaya ringspot virus, Paprika mild mottle virus, Pariacoto virus, Parsnip yellow fleck virus, Patchouli mild mosaic virus, Pea early browning virus, Pea enation mosaic virus-1, Pea enation mosaic virus-2, Pea seed-borne mosaic virus, Peanut clump virus, Peanut mottle virus, Peanut stunt virus, Pear latent virus, Pelargonium zonate spot virus, Pepino mosaic virus, Pepper mild mottle virus, Pepper mottle virus, Pepper ringspot virus, Perina nuda picorna-like virus, Peru tomato mosaic virus, Pestivirus Giraffe-1, Pestivirus Reindeer-1, Pestivirus type 1, Pestivirus type 2, Pestivirus type 3, Physalis mottle virus, Plantago asiatica mosaic virus, Plautia stali intestine virus, Plum pox virus, Poinsettia mosaic virus, Poliovirus, Porcine enteric calicivirus, Porcine enterovirus A, Porcine enterovirus B, Porcine epidemic diarrhea virus, Porcine reproductive and respiratory syndrome virus, Porcine teschovirus 1, Potato aucuba mosaic virus, Potato leafroll virus, Potato mop-top virus, Potato virus A, Potato virus M, Potato virus V, Potato virus X, Potato virus Y, Pothos latent virus, Powassan virus, Prunus necrotic ringspot virus, Pseudomonas phage PP7, Rabbit hemorrhagic disease virus, Raspberry bushy dwarf virus, Red clover mottle virus, Red clover necrotic mosaic virus, Rhopalosiphum padi virus, Ribgrass mosaic virus, Rice tungro spherical virus, Rice yellow mottle virus, Rio Bravo virus, Ross River virus, Rubella virus, Rupestris stem pitting associated virus-1, Ryegrass mosaic virus, Ryegrass mottle virus, Sacbrood virus, Saccharomyces cerevisiae narnavirus 20S RNA , Saccharomyces cerevisiae narnavirus 23S RNA, Saguaro cactus virus, Salmon pancreas disease virus, SARS coronavirus, Satsuma dwarf virus, Scallion mosaic virus, Scallion virus X, Semliki forest virus, Sesbania mosaic virus, Shallot virus X, Simian hemorrhagic fever virus, Simian picornavirus 1, Sindbis virus, Sleeping

disease virus, Soil-borne cereal mosaic virus, Soil-borne wheat mosaic virus, Sorghum chlorotic spot virus, Sorghum mosaic virus, Southern bean mosaic virus, Southern cowpea mosaic virus, Soybean dwarf virus, Soybean mosaic virus, Spinach latent virus, Spring beauty latent virus, Squash mosaic virus, Strawberry mild yellow edge virus, Strawberry mottle virus, Striped Jack nervous necrosis virus, Subterranean clover mottle virus, Sugarcane mosaic virus, Sugarcane striate mosaic associated virus, Sugarcane yellow leaf virus, Sweet clover necrotic mosaic virus, Sweet potato chlorotic stunt virus, Sweet potato feathery mottle virus, Sweet potato mild mottle virus, Tamana bat virus, Taura syndrome virus, Theilovirus, Tick-borne encephalitis virus, Tobacco bushy top virus, Tobacco etch virus, Tobacco mild green mosaic virus, Tobacco mosaic virus, Tobacco necrosis virus A, Tobacco necrosis virus D, Tobacco rattle virus, Tobacco streak virus, Tobacco vein mottling virus, Tomato aspermy virus, Tomato black ring virus, Tomato bushy stunt virus, Tomato mosaic virus, Tomato ringspot virus, Transmissible gastroenteritis virus, Triatoma virus, Tulare apple mosaic virus, Tulip virus X, Turkey astrovirus, Turnip crinkle virus, Turnip mosaic virus, Turnip rosette virus, Turnip vein-clearing virus, Turnip yellow mosaic virus, Turnip yellows virus, Venezuelan equine encephalitis virus, Vesicular exanthema of swine virus, Walrus calicivirus, West Nile virus, Western equine encephalomyelitis virus, Wheat streak mosaic virus, Wheat yellow mosaic virus, White clover mosaic virus, Wild potato mosaic virus, Yam mosaic virus, Yellow fever virus, Youcai mosaic virus, Zucchini green mottle mosaic virus and Zucchini yellow mosaic virus. Preferably, the 3' UTR is that of a positive strand single-stranded plant virus having no DNA stage, and can be, for example, a 3' UTR of a Cowpea chlorotic mottle virus, a 3' UTR of a Brome mosaic bromovirus, a 3' UTR of a Lettuce mosaic virus, or a 3' UTR of a Cucumber mosaic virus.

[0055] Preferably, the 3' UTR is the 3' UTR of a Cowpea chlorotic mottle virus. A DNA copy of a Cowpea chlorotic mottle virus 3' UTR can comprise, for example, the sequence:

AGTGCCCGCTGAAGAGCGTTACACTAGTGTGGCCTACTTGAAGGCTAGTTATAACCG
TTTCTTTAAACGGTAATCGTTGTTGAAACGTCTTCCTTTTACAAGAGGATTGAGCTGC
CCTTGGGTTTTACTCCTTGAACCCTTCGGAAGAACTCTTTGGAGTTCGTACCAGTACC
TCACATAGTGAGGTAATAAGACTGGTGGGCAGCGCCTAGTCGAAAGACTAGGTGAT
CTCTAAGGAGACC (SEQ ID NO: 8).

[0056] An RNA copy of the 3' UTR can comprise, for example, the sequence:

AGUGCCCGCUGAAGAGCGUUACACUAGUGUGGCCUACUUGAAGGCUAGUUAUAA
CCGUUUCUUUAAACGGUAAUCGUUGUUGAAACGUCUUCCUUUUACAAGAGGAUU
GAGCUGCCCUUGGGUUUUACUCCUUGAACCCUUCGGAAGAACUCUUUGGAGUUCG
UACCAGUACCUCACAUAGUGAGGUAAUAAGACUGGUGGGCAGCGCCUAGUCGAA
AGACUAGGUGAUCUCUAAGGAGACC (SEQ ID NO: 9).

[0057] A DNA copy of the complement of the 3' UTR can comprise, for example, the sequence:

GGTCTCCTTAGAGATCACCTAGTCTTTCGACTAGGCGCTGCCCACCAGTCTTATTACC
TCACTATGTGAGGTACTGGTACGAACTCCAAAGAGTTCTTCCGAAGGGTTCAAGGAG
TAAAACCCAAGGGCAGCTCAATCCTCTTGTAAAAGGAAGACGTTTCAACAACGATT
ACCGTTTAAAGAAACGGTTATAACTAGCCTTCAAGTAGGCCACACTAGTGTAACGCT
CTTCAGCGGGCACT (SEQ ID NO: 10).

[0058] An RNA copy of the complement of the 3' UTR can comprise, for example, the sequence:

GGUCUCCUUAGAGAUCACCUAGUCUUUCGACUAGGCGCUGCCCACCAGUCUUAUU
ACCUCACUAUGUGAGGUACUGGUACGAACUCCAAAGAGUUCUUCCGAAGGGUUC
AAGGAGUAAAACCCAAGGGCAGCUCAAUCCUCUUGUAAAAGGAAGACGUUUCAA
CAACGAUUACCGUUUAAAGAAACGGUUAUAACUAGCCUUCAAGUAGGCCACACU
AGUGUAACGCUCUUCAGCGGGCACU (SEQ ID NO: 11).

[0059] In another aspect, the invention is directed to a host cell comprising the recombinant DNA transgene. The host cell can be any eukaryotic cell, preferably a plant cell. The plant host cell can be a dicotyledonous plant host cell or a monocotyledonous plant host cell. The plant host cell can be a crop plant host cell. In preferred embodiments, the plant cell is a dicotyledonous plant host cell, preferably a *Nicotiana* plant host cell, more preferably a *Nicotiana bentamiana* host plant cell.

[0060] In another embodiment, the invention is directed to a recombinant RNA molecule, wherein the recombinant RNA molecule comprises, in 5' to 3' direction, an anti-sense coding sequence for a heterologous polypeptide, an anti-sense IRES, and a 3' UTR of a positive strand single-stranded RNA virus. The anti-sense coding sequence for a heterologous polypeptide of the recombinant RNA can correspond in sequence to the anti-sense coding sequence for a heterologous polypeptide of the recombinant DNA transgene described above.

[0061] The methods disclosed in the present invention comprise inoculating, infecting or tranfecting a transgenic host cell or organism with a positive strand single-stranded RNA virus having no DNA stage in order to stimulate or activate the formation of a complementary strand of the transgene. The inoculating, infecting or transfecting can be by any inoculating, infection or transfection method known in the art. The positive strand single-stranded RNA virus having no DNA stage that can be used to stimulate or activate the formation of the RNA complement of the

recombinant RNA can be a plant virus or an animal virus, a portion thereof, or a nucleic acid thereof. Non-limiting examples of single-stranded RNA positive-strand plant viruses having no DNA stage include: Allexivirus, such as, for example, Garlic virus A, Garlic virus B, Garlic virus C, Garlic virus D, Garlic virus E, Garlic virus X, Shallot virus X; Benyvirus, such as, for example, Beet necrotic yellow vein virus, Beet soil-borne mosaic virus (BSBMV); Bromoviridae, such as, for example, Alfamovirus, such as, for example, Alfalfa mosaic virus; Bromovirus, such as, for example, Broad bean mottle virus, Brome mosaic virus, Cowpea chlorotic mottle virus, Spring beauty latent virus; Cucumovirus, such as, for example, Cucumber mosaic virus (cucumber mosaic cucumovirus), Peanut stunt virus, Tomato aspermy virus; Ilarvirus, such as, for example, American plum line pattern virus, Tobacco streak virus, Asparagus virus 2, Citrus leaf rugose virus, Citrus variegation virus, Elm mottle virus, Tulare apple mosaic virus, Apple mosaic virus, Prunus necrotic ringspot virus, Prune dwarf virus, Spinach latent virus, Lilac ring mottle virus, Hydrangea mosaic virus 8; Oleavirus, such as, for example, Olive latent virus 2, Pelargonium zonate spot virus; Caliciviridae, such as, for example, Capillovirus, such as, for example, Apple stem grooving virus, Citrus tatter leaf virus, Cherry virus A; Carlavirus, such as, for example, Aconitum latent virus, Alfalfa latent carlavirus, Blueberry scorch virus, Carnation latent virus, Chrysanthemum virus B, Cowpea mild mottle virus, Garlic common latent virus, Garlic latent virus, Garlic latent virus E29-6, Garlic virus 1, Helenium virus S, Hop latent virus, Hop mosaic virus, Kalanchoe latent virus, Lily latent virus (LiLV), Lily symptomless virus (LSV), Narcissus carlavirus, Pea streak virus, Poplar mosaic virus, Poplar mosaic virus (ATCC PV257), Potato latent virus, Potato rough dwarf virus, Potato virus M, Potato virus S, Shallot latent virus, Sugarcane striate mosaic virus, unidentified Verbena-infecting Carlavirus; Closteroviridae, such as, for example, Ampelovirus, such as, for

example, Grapevine leafroll-associated virus 1, Grapevine leafroll-associated virus 3, Grapevine leafroll-associated virus 4, Grapevine leafroll-associated virus 5, Grapevine leafroll-associated virus 6, Grapevine leafroll-associated virus 8, Little cherry virus 2, Pineapple mealybug wilt-associated virus 1, Pineapple mealybug wilt-associated virus 2, Plum bark necrosis stem pitting virus; Closterovirus, such as, for example, Apricot stem pitting associated virus, Beet yellow stunt virus, Beet yellows virus, Citrus tristeza virus, Grapevine leafroll-associated virus 2, Grapevine rootstock stem lesion associated virus, Olive leaf yellowing associated virus; Crinivirus, such as, for example, Beet pseudo-yellows virus, Cucumber yellows virus, Cucurbit yellow stunting disorder virus, Lettuce infectious yellows virus, Potato yellow vein virus, Strawberry pallidosis associated virus, Sweet potato chlorotic stunt virus, Tomato chlorosis virus, Tomato infectious chlorosis virus; unassigned species in the family Closteroviridae, such as, for example, Grapevine leafroll-associated virus 7, Little cherry virus 1; Comoviridae, such as, for example, Comovirus, such as, for example, Andean potato mottle virus, Bean pod mottle virus, Bean rugose mosaic virus, Cowpea mosaic virus, Cowpea severe mosaic virus, Red clover mottle virus, Squash mosaic virus; Fabavirus, such as, for example, Broad bean wilt virus, Broad bean wilt virus 1, Broad bean wilt virus 2, Patchouli mild mosaic virus; Nepovirus, such as, for example, Apricot latent ringspot virus, Grapevine fanleaf virus, Arabis mosaic virus, Raspberry ringspot virus, Raspberry ringspot virus (strain S), Tobacco ringspot virus, Artichoke italian latent virus, Beet ringspot virus, Cycas necrotic stunt virus, Grapevine chrome mosaic virus, Olive latent ringspot virus, Tomato black ring virus, Blackcurrant reversion virus, Blueberry leaf mottle virus, Cherry leaf roll virus, Chicory yellow mottle virus, Peach rosette mosaic virus, Tomato ringspot virus;, unclassified Comoviridae, such as, for example, Cherry rasp leaf virus; Foveavirus, such as, for example, African oil palm ringspot virus, Apple stem pitting virus,

Banana mild mosaic virus, Cherry green ring mottle virus, Cherry necrotic rusty mottle virus, Peach asteroid spot virus, Peach sooty ringspot virus, Prunus mume foveavirus, Rupestris stem pitting-associated virus; Furovirus, such as, for example, Chinese wheat mosaic virus, Nicotiana velutina mosaic virus, Oat golden stripe virus, Soil-borne cereal mosaic virus, Soil-borne wheat mosaic virus, Sorghum chlorotic spot virus; Hordeivirus, such as, for example, Barley stripe mosaic virus, Lychnis ringspot virus, Poa semilatent virus; Idaeovirus, such as, for example, Raspberry bushy dwarf virus; Luteoviridae, such as, for example, Enamovirus, such as, for example, Pea enation mosaic virus; Luteovirus, such as, for example, Barley yellow dwarf virus, Bean leafroll virus, Carrot red leaf virus, Chickpea stunt disease associated virus, Groundnut rosette assistor virus, Soybean dwarf virus, Tobacco vein-distorting virus; Polerovirus, such as, for example, Beet chlorosis virus, Beet mild yellowing virus, Beet western yellows virus, Cereal yellow dwarf virus-RPS, Cereal yellow dwarf virus-RPV, Cucurbit aphid-borne yellows virus, Potato leafroll virus, Tobacco vein distorting polerovirus, Turnip yellows virus; Unassigned Luteoviridae, such as, for example, Sugarcane yellow leaf virus; Marafivirus, such as, for example, Bermuda grass etched-line virus, Maize rayado fino virus, Oat blue dwarf virus, Poinsettia mosaic virus; Pecluvirus, such as, for example, Indian peanut clump virus, such as, for example, Indian peanut clump virus D, Indian peanut clump virus H, Indian peanut clump virus L; Peanut clump virus, such as, for example, Peanut clump virus B, Peanut clump virus M, Peanut clump virus N, Peanut clump virus Ni; Pomovirus, such as, for example, Beet soil-borne virus, Beet virus Q, Broad bean necrosis virus, Potato mop-top virus; Potexvirus, such as, for example, Alternanthera potexvirus, Bamboo mosaic virus, Cactus virus X, Cassava common mosaic virus, Clover yellow mosaic virus, Cymbidium mosaic virus, Foxtail mosaic virus, Hydrangea ringspot virus, Lily virus X, Narcissus mosaic virus, Papaya mosaic virus, Pepino

mosaic virus, Plantago asiatica mosaic potexvirus, Plantago asiatica mosaic virus, Potato aucuba mosaic virus, Potato virus X, Scallion virus X, Strawberry mild yellow edge virus, Tulip virus X, White clover mosaic virus; Potyviridae, such as, for example, Bymovirus, such as, for example, Barley mild mosaic virus, Barley yellow mosaic virus, Oat mosaic virus, Rice necrosis mosaic virus, Wheat spindle streak mosaic virus, Wheat yellow mosaic virus; Ipomovirus, such as, for example, Cassava brown streak virus, Sweet potato mild mottle virus; Macluravirus, such as, for example, Maclura mosaic virus, such as, for example, Cardamom mosaic virus, Indian cardamom mosaic virus, Narcissus latent virus; Potyvirus, such as, for example, Alpinia mosaic virus, Apium virus Y, Artichoke latent potyvirus, Banana bract mosaic virus, Bean black root virus, Bean common mosaic necrosis virus; Bean common mosaic virus, such as, for example, Azuki bean mosaic virus, Blackeye cowpea mosaic virus, Dendrobium mosaic virus, Peanut stripe virus, Bean yellow mosaic virus, Beet mosaic virus, Brome streak mosaic potyvirus, Calanthe mild mosaic potyvirus, Carnation vein mottle virus, Carrot thin leaf virus, Carrot virus Y, Celery mosaic virus, Celery yellow mosaic virus, Ceratobium mosaic potyvirus, Chilli vein-banding mottle virus, Chinese narcissus potyvirus, Clitoria virus Y, Clover yellow vein virus, Cocksfoot streak virus, Colombian datura potyvirus, Cowpea aphid-borne mosaic virus, Crotalaria mosaic potyvirus, Cucurbit yellows-associated virus, Cypripedium virus Y, Dasheen mosaic virus, Dioscorea dumentorum virus, Diurus virus Y, Endive necrotic mosaic virus, Garlic mite-borne mosaic virus, Garlic mosaic virus, Garlic potyvirus 1, Garlic virus 2, Gloriosa stripe mosaic virus, Hibbertia virus Y, Iranian Johnson grass mosaic virus, Iris mild mosaic virus, Iris severe mosaic virus, Japanese hornwort mosaic virus, Japanese yam mosaic virus, Johnsongrass mosaic virus, Leek yellow stripe potyvirus, Lettuce mosaic virus, Lily mottle virus, Lycoris mild mottle virus, Maize dwarf mosaic virus, Moroccan watermelon mosaic virus, Narcissus late

season yellow virus, Narcissus yellow stripe virus, Onion yellow dwarf virus, Ornithogalum mosaic virus, Ornithogalum virus 2, Ornithogalum virus 3, Papaya leaf-distortion mosaic potyvirus, Papaya ringspot virus, Passion fruit woodiness virus, Pea seed-borne mosaic virus, Peanut chlorotic blotch virus, Peanut mottle virus, Pennisetum flaccidum mosaic virus, Pepper mottle virus, Pepper severe mosaic virus, Pepper vein banding virus, Pepper yellow mosaic virus, Peru tomato mosaic virus, Petunia flower mottle virus, Pleione virus Y, Plum pox virus, Potato virus A, Potato virus V, Potato virus Y, Pterostylis virus Y, Rembrandt tulip-breaking virus, Rhopalanthe virus Y, Sarcochilus virus Y, Sesame mosaic potyvirus, Shallot potyvirus, Shallot yellow stripe virus, Sorghum mosaic virus, South African passiflora virus, Soybean mosaic virus, Sugarcane mosaic virus, Sugarcane streak mosaic virus, Sunflower chlorotic mottle virus, Sunflower chlorotic spot virus, Sunflower mosaic virus, Sweet potato feathery mottle virus, Sweet potato G virus, Sweet potato latent virus, Sweet potato mild speckling potyvirus, Sweet potato virus Y, Tamarillo mosaic virus, Tobacco etch virus, Tobacco vein banding mosaic virus, Tobacco vein mottling virus, Tuberose mild mosaic virus, Tulip band-breaking virus, Tulip breaking virus, Tulip mosaic virus, Tulip top-breaking virus, Turnip mosaic virus, Vanilla mosaic virus, Watermelon bud necrosis virus, Watermelon leaf mottle virus; Watermelon mosaic virus, such as, for example, Vanilla necrosis virus, Welsh onion yellow stripe virus, Wild potato mosaic virus, Wisteria vein mosaic virus, Yam mild mosaic virus, Yam mosaic virus, Zantedeschia mosaic virus, Zea mosaic virus, Zucchini yellow mosaic virus; Rymovirus, such as, for example, Agropyron mosaic virus, Hordeum mosaic virus, Oat necrotic mottle virus, Ryegrass mosaic virus; Tritimovirus, such as, for example, Brome streak mosaic virus, Wheat streak mosaic virus; unclassified Potyviridae, such as, for example, Chinese yam necrotic mosaic virus, Cucumber vein yellowing virus, Scallion mosaic virus, Spartina mottle virus, Tomato mild

mottle virus; Sequiviridae, such as, for example, SDV-like viruses, such as, for example, Apple latent spherical virus, Citrus mosaic virus, Navel orange infectious mottling virus, Satsuma dwarf virus, Strawberry latent ringspot virus, Strawberry mottle virus; Sequivirus, such as, for example, Parsnip yellow fleck virus; Waikavirus, such as, for example, Maize chlorotic dwarf virus, Rice tungro spherical virus; Sobemovirus, such as, for example, Cocksfoot mottle virus, Lucerne transient streak virus, Rice yellow mottle virus, Ryegrass mottle virus, Sesbania mosaic virus, Southern bean mosaic virus, Southern cowpea mosaic virus, Subterranean clover mottle virus, Turnip rosette virus; Tetraviridae, such as, for example, Betatetravirus, such as, for example, Nudaurelia capensis beta virus; Omegatetravirus, such as, for example, Nudaurelia capensis omega virus; unclassified Tetraviridae, such as, for example, Helicoverpa armigera stunt virus, Providence virus, Thosea asigna virus; Tobamovirus, such as, for example, Chinese Rape Mosaic Virus, Crucifer tobamovirus, Cucumber fruit mottle mosaic virus, Cucumber green mottle mosaic virus, Frangipani mosaic virus, Hibiscus virus S, Kyuri green mottle mosaic virus, Obuda pepper virus, Odontoglossum ringspot virus, Paprika mild mottle virus, Pepper mild mottle virus, Ribgrass mosaic virus, Sunn-hemp mosaic virus, Tobacco mild green mosaic virus, Tobacco mosaic virus, Tomato mosaic virus, Turnip vein-clearing virus, Youcai mosaic virus, Zucchini green mottle mosaic virus; Tobravirus, such as, for example, Pea early browning virus, Pepper ringspot virus, Tobacco rattle virus; Tombusviridae, such as, for example, Aureusvirus, such as, for example, Pothos latent virus; Avenavirus, such as, for example, Oat chlorotic stunt virus; Carmovirus, such as, for example, Calibrachoa mottle virus, Cardamine chlorotic fleck virus, Carnation mottle virus, Cowpea mottle virus, Elderberry latent virus, Galinsoga mosaic virus, Hibiscus chlorotic ringspot virus, Japanese iris necrotic ring virus, Melon necrotic spot virus, Pelargonium flower break virus, Saguaro cactus virus, Turnip crinkle virus; Dianthovirus,

such as, for example, Carnation ringspot virus, Dianthovirus RVX1, Red clover necrotic mosaic virus, Sweet clover necrotic mosaic virus; Machlomovirus, such as, for example, Maize chlorotic mottle virus; Necrovirus, such as, for example, Beet black scorch virus, Leek white stripe virus, Olive latent virus 1; Panicovirus, such as, for example, Panicum mosaic virus; Tombusvirus, such as, for example, Artichoke mottled crinkle virus, Carnation Italian ringspot virus, Cucumber Bulgarian virus, Cucumber necrosis virus, Cymbidium ringspot virus, Grapevine Algerian latent virus, Lettuce necrotic stunt virus, Moroccan pepper virus, Pear latent virus, Pelargonium leaf curl virus, Tomato bushy stunt virus; unclassified Tombusviridae, such as, for example, Cucumber leaf spot virus, Maize necrotic streak virus, Pelargonium chlorotic ring pattern virus, Pelargonium line pattern virus, Pelargonium ringspot virus; Trichovirus, such as, for example, Apple chlorotic leaf spot virus, Apricot trichovirus, Cherry mottle leaf virus, Grapevine berry inner necrosis virus, Peach mosaic virus, Potato trichovirus T; Tymovirus, such as, for example, Andean potato latent virus, Belladonna mottle virus, Cacao yellow mosaic virus, Chayote mosaic tymovirus, Clitoria yellow vein virus, Desmodium yellow mottle tymovirus, Dulcamara mottle virus, Eggplant mosaic virus, Erysimum latent virus, Kennedya yellow mosaic virus, Okra mosaic tymovirus, Ononis yellow mosaic virus, Passion fruit yellow mosaic virus, Petunia vein banding virus, Physalis mottle virus, Turnip yellow mosaic virus, Wild cucumber mosaic virus; Umbravirus, such as, for example, Carrot mottle mimic virus, Groundnut rosette virus, Pea enation mosaic virus-2, Tobacco bushy top virus, Tobacco mottle virus; Vitivirus, such as, for example, Grapevine virus A, Grapevine virus B, Grapevine virus D, Heracleum latent virus; unclassified single-stranded RNA positive-strand viruses, such as, for example, Acyrthosiphon pisum virus, Apricot latent virus, Beet western yellows ST9 associated virus, Botrytis virus F, Citrus leaf blotch virus, Euprosterna elaeasa virus, Grapevine fleck virus, Indian citrus ringspot

virus, Oyster mushroom spherical virus, Pear vein yellows-associated virus, and Sugarcane striate mosaic associated virus.

[0062] Non-limiting examples of single-stranded RNA positive-strand animal viruses having no DNA stage include: Astroviridae, such as, for example, Astrovirus, such as, for example, Feline astrovirus, Human astrovirus, Mink astrovirus, Ovine astrovirus, Porcine astrovirus, Turkey astrovirus, Astrovirus sp., Avian nephritis virus; Caliciviridae, such as, for example, Lagovirus, such as, for example, European brown hare syndrome virus, Rabbit hemorrhagic disease virus, Norovirus, such as, for example, Bovine enteric calici-like virus, Maryland calicivirus 6, Minireovirus, Murine norovirus 1, Norwalk virus, such as, for example, Camberwell virus, Chiba virus, Chitta virus, Desert Shield virus, Hawaii calicivirus, Human calicivirus genogroup 1, Norwalk virus, Lordsdale virus, Maryland calicivirus 1, Norwalk-like virus, Norwalk-like virus genogroup 2, Small round structured virus, Snow Mountain virus, Southampton virus; Oyster norovirus, Saratoga calicivirus 7, Swine calicivirus, Sapovirus, such as, for example, Human calicivirus strain HuCV/Potsdam/2000/DEU, Manchester virus, Mink enteric calicivirus, Porcine enteric calicivirus, Sapporo virus, Toronto calicivirus 24, Vesivirus, such as, for example, Feline calicivirus, FCV-like Calicivirus, Unassigned Veriviruses, such as, for example, Canine calicivirus, Mink calicivirus; Walrus calicivirus, Vesicular exanthema of swine virus, such as, for example, Bovine calicivirus, Bovine Calicivirus Bos-2, Cetacean calicivirus, Primate calicivirus, Reptile calicivirus; San Miguel sea lion virus, such as, for example, San Miguel sea lion virus 13, San Miguel sea lion virus 2, San Miguel sea lion virus 6, San Miguel sea lion virus 1, San Miguel sea lion virus 4, Skunk calicivirus, Vesicular exanthema of swine virus A48, VESV-like calicivirus; unclassified Caliciviridae, such as, for example, Calicivirus strain CV23-OH, Calicivirus strain NB, Human calicivirus strain A141, Chiba virus,

Chitta virus, Desert Shield virus, Hawaii calicivirus, Human calicivirus genogroup 1, Norwalk virus, Lordsdale virus, Maryland calicivirus 1, Norwalk-like virus, Norwalk-like virus genogroup 2, Small round structured virus, Snow Mountain virus, Southampton virus, Cricket paralysis-like viruses, such as, for example; Acute bee paralysis virus, Aphid lethal paralysis virus, Black queen cell virus, Cricket paralysis virus, Drosophila C virus, Himetobi P virus, Kashmir bee virus, Plautia stali intestine virus, Rhopalosiphum padi virus, Taura syndrome virus, Triatoma virus, Flaviviridae, such as, for example, Flavivirus (arboviruses group B), such as, for example, Cell fusing agent virus, Dengue virus group, Japanese encephalitis virus group, Modoc virus group, mosquito-borne viruses, Ntaya virus group, Rio Bravo virus group, tick-borne encephalitis virus group, Tyuleniy virus group, Uganda S virus group, Yellow fever virus group, unclassified Flavivirus, Hepacivirus, such as, for example, Hepatitis C virus; Pestivirus, such as, for example, Bovine viral diarrhea virus genotype 2 (BVDV-2), Pestivirus type 1, Pestivirus type 2, Pestivirus type 3, unclassified Pestivirus, unclassified Flaviviridae, such as, for example, Douroucouli hepatitis GB virus A, GBV-A-like virus, GBV-C/HGV group, Hepatitis GB virus A, Hepatitis GB virus B, Marmoset hepatitis GB virus A, Turkey meningoencephalitis virus, Nidovirales, such as, for example; Arteriviridae, such as, for example, Arterivirus, such as, for example, Equine arteritis virus, Lactate dehydrogenase-elevating virus, Porcine reproductive and respiratory syndrome virus, Lelystad virus, Simian hemorrhagic fever virus; Coronaviridae, such as, for example, Coronavirus, such as, for example, Avian infectious bronchitis virus, Avian infectious laryngotracheitis virus, Enteric coronavirus, Equine coronavirus, Group 1 species, such as, for example, Canine coronavirus, such as, for example, Canine enteric coronavirus (strain INSAVC-1), Canine enteric coronavirus (strain K378), Feline coronavirus, such as, for example, Feline enteric coronavirus (strain 79-1683), Feline infectious peritonitis virus (FIPV),

Human coronavirus 229E, Porcine epidemic diarrhea virus, Transmissible gastroenteritis virus, such as, for example, Porcine respiratory coronavirus, Porcine transmissible gastroenteritis coronavirus, Group 2 species, such as, for example, Bovine coronavirus, Chicken enteric coronavirus, Human coronavirus OC43, Murine hepatitis virus, Porcine hemagglutinating encephalomyelitis virus, Puffinosis virus, Rat coronavirus, such as, for example, Rat coronavirus (strain 681), Rat sialodacryoadenitis coronavirus, Group 3 species, such as, for example, Turkey coronavirus, Human enteric coronavirus 4408, SARS coronavirus, Torovirus, such as, for example, Berne virus, Bovine torovirus, Breda virus, Human torovirus, Roniviridae; Okavirus such as, for example, Gill-associated virus, Yellow head virus, Nodaviridae, such as, for example, Alphanodavirus, such as, for example, Black beetle virus, Boolarra virus, Flock house virus, Nodamura virus, Pariacoto virus, Betanodavirus, such as, for example, Atlantic cod nervous necrosis virus, Atlantic halibut nodavirus, Barfin flounder nervous necrosis virus, Dicentrarchus labrax encephalitis virus, Dragon nervous necrosis virus, Epinephelus coioides nervous necrosis virus, Epinephelus tauvina nervous necrosis virus, Guppy nervous necrosis virus, Japanese flounder nervous necrosis virus, Malabaricus nervous necrosis virus, Redspotted grouper nervous necrosis virus, Striped Jack nervous necrosis virus, Tiger puffer nervous necrosis virus, Umbrina cirrosa nodavirus, Picornaviridae, such as, for example, Aphthovirus, such as, for example, Equine rhinitis A virus, Foot-and-mouth disease virus, Cardiovirus, such as, for example, Encephalomyocarditis virus; Mengo virus, Porcine encephalomyocarditis virus, Theilovirus, such as, for example, Theiler's encephalomyelitis virus, Enterovirus, such as, for example, Bovine enterovirus, Coxsackievirus, Echovirus, Human echovirus 1, Human enterovirus A, Human enterovirus B, Human enterovirus C, Human enterovirus D, Human enterovirus E, Poliovirus, Porcine enterovirus A, Porcine enterovirus B, Sheep enterovirus,

Erbovirus, such as, for example, Equine rhinitis B virus, Hepatovirus, such as, for example, Hepatitis A virus, such as, for example, Human hepatitis A virus, Simian hepatitis A virus, Kobuvirus, such as, for example, Aichi virus, Bovine kobuvirus, Parechovirus, such as, for example, Human parechovirus, Ljungan virus, Porcine enterovirus 11, Porcine enterovirus 2, Porcine enterovirus 3, Porcine enterovirus 4, Porcine enterovirus 5, Porcine enterovirus 6, Porcine enterovirus 7, Porcine enterovirus J1, Porcine enterovirus J10, Porcine enterovirus J2, Porcine enterovirus J3, Porcine enterovirus J4, Porcine enterovirus J5, Porcine enterovirus J6, Porcine enterovirus J7, Porcine enterovirus J9, Rhinovirus (common cold viruses), such as, for example, Equine rhinovirus 3, Human rhinovirus A, such as, for example; Human rhinovirus 11, Human rhinovirus 15, Human rhinovirus 16, Human rhinovirus 1A, Human rhinovirus 1B, Human rhinovirus 2, Human rhinovirus 21, Human rhinovirus 29, Human rhinovirus 36, Human rhinovirus 39, Human rhinovirus 49, Human rhinovirus 50, Human rhinovirus 58, Human rhinovirus 62, Human rhinovirus 65, Human rhinovirus 7, Human rhinovirus 85, Human rhinovirus 89, Human rhinovirus 9, Human rhinovirus B, such as, for example; Teschovirus, such as, for example, Human rhinovirus 14, Human rhinovirus 3, Human rhinovirus 72, Porcine teschovirus, unclassified Picornaviridae, such as, for example, Avian encephalomyelitis virus, Clethrionomys glareolus picornavirus, Maus-Elberfeld virus, Picornaviridae strain 62.3, Picornaviridae strain 62.4, Picornaviridae strain 62.8, Picornaviridae strain 62.9, Picornaviridae strain IG.26, Simian picornavirus 1, Simian picornavirus 10, Simian picornavirus 11, Simian picornavirus 12, Simian picornavirus 13, Simian picornavirus 15, Simian picornavirus 17, Simian picornavirus 18, Simian picornavirus 2, Simian picornavirus 3, Simian picornavirus 4, Simian picornavirus 5, Simian picornavirus 6, Simian picornavirus 7, Simian picornavirus 7', Simian picornavirus 8, Simian picornavirus 9, Simian picornavirus strain N125, Simian

picornavirus strain N203, Tetraviridae, such as, for example, Betatetravirus, such as, for example, Nudaurelia capensis beta virus, Omegatetravirus, such as, for example, Nudaurelia capensis omega virus, unclassified Tetraviridae, such as, for example, Helicoverpa armigera stunt virus, Providence virus, Thosea asigna virus, Togaviridae, such as, for example, Alphavirus (arboviruses group A), such as, for example, BFV complex, such as, for example; Barmah Forest virus, EEEV complex, such as, for example, Eastern equine encephalitis virus, Igbo Ora virus, Karelian fever virus, Middelburg virus, NDUV complex, such as, for example, Ndumu virus, Salmon pancreas disease virus, Seal louse virus, SFV complex, such as, for example, Bebaru virus, Chikungunya virus, Getah virus, Mayaro virus, Me Tri virus, O'nyong-nyong virus, Ross River virus, Sagiyama virus, Semliki forest virus, Una virus, Sleeping disease virus, Trocara virus, VEEV complex, such as, for example, 71D1252 virus, 78V3531 virus, Ag80-663 virus, Bijou Bridge virus, Cabassou virus, Mucambo virus, Tonate virus, Pixuna virus, Venezuelan equine encephalitis virus, WEEV complex, such as, for example, Aura virus, Fort Morgan virus, Buggy Creek virus, Highlands J virus, Sindbis virus; such as, for example, Babanki virus, Kyzylagach virus, Ockelbo virus, Sindbis virus (strain HRSP), Sindbis virus (wild type SB derived from strain AR339), Sindbis-like virus, Sindbis-like virus YN87448, Western equine encephalomyelitis virus, Whataroa virus, Rubivirus, such as, for example, Rubella virus.

[0063] Preferably, the positive strand single-stranded RNA virus or nucleic acid thereof used to stimulate or activate the formation of the RNA complement of the recombinant RNA is a plant virus, preferably a Cowpea chlorotic mosaic virus (CCMV), a Brome mosaic bromovirus (BMV), a Lettuce mosaic virus, a Tobacco etch virus, a Tobacco vein mottle virus, a Pepper mottle virus, or a Tomato aspermy virus. More preferably, the virus is a CCMV or a BMV. The RNA virus cannot be a Cucumber mosaic virus when the 3' UTR of the transgene is that of

Lettuce mosaic virus. It is expected that many different species of positive strand single-stranded RNA virus or nucleic acid thereof can encode polypeptides (such as, for example, an RNA-dependent RNA polymerase) that comprise a replication complex that recognizes a 3' UTR of a recombinant RNA as described herein. Preferably, the virus or nucleic acid thereof used to stimulate or activate the formation of the RNA complement of the recombinant RNA can be the same virus as the source of the 3' UTR of the recombinant RNA or transgene.

[0064] The method as described herein, therefore, comprises providing a transgenic cell or organism comprising a recombinant DNA, the recombinant DNA comprising a promoter operably linked, in 5' to 3' direction, the complement of a coding sequence for a heterologous polypeptide, the complement of an IRES, and a 3' UTR of an RNA virus; infecting the cell or organism with a virus or a portion thereof, or transfecting the cell or organism with an RNA or a cDNA of an RNA virus or portion thereof, wherein the portion provides sequences encoding viral polypeptide components of a replication complex, such as, for example, an RNA-dependent RNA polymerase.

[0065] The kit as described herein comprises a DNA vector comprising, in the 5' to 3' direction, a promoter, at least one site for incorporation of coding sequence of a heterologous polypeptide in an antisense orientation, an anti-IRES, and a 3' UTR of a positive strand single-stranded RNA virus, and packaging. A user of the kit can, for example, incorporate coding sequence for a heterologous polypeptide into the DNA vector such that transcription of the vector would yield a transcript comprising, in the 5' to 3' direction, the complement of the coding sequence, the complement of the IRES, and the 3' UTR. The kit can further comprise a positive strand single-stranded RNA virus or nucleic acid thereof that, upon infection or transfection, would support the formation of an RNA complementary to the recombinant RNA. The kit can

further comprise a host organism for growing the vector, such as, for example, transformation-competent *E. coli*. In some aspects, the kit can further comprise laboratory disposables such as, for example, plastic tubes and pipette tips. The kit can further comprise instructions and packaging.

EXAMPLES

[0066] Example 1

[0067] This example illustrates recombination of complementary copies of viral transgenes during viral replication.

[0068] Cowpea chlorotic mottle bromovirus (CCMV) was used initially to demonstrate that transgenic viral gene transcripts are available in the cytoplasm for recombination with a replicating virus (Greene and Allison, *Science* 263: 1423-1425, 1994). In these experiments, transgenic transcripts included part of the viral coat gene as well as a complete CCMV 3' UTR. However, when a portion or all of the 3' UTR was deleted from the transgenic viral gene transcript, viral recombination was below detection limits, suggesting that recombination of a transcript of a viral transgene requires the presence of an intact 3' UTR in the transcript. Without being limited by theory, the observations suggested that the presence of a complete 3' UTR enhances the stability of a transcript of the transgene in the cytoplasm, thereby prolonging the transcript's availability for recombination. These observations raise the possibility that the complete 3' UTR and its replication complex binding site may be recognized by a replication complex of a challenging virus, and a complementary copy of a transgenic transcript capable of contributing to recombination events may be synthesized in the cytoplasm. Recombination

events could involve both an original transgenic transcript and its complementary copy, and could occur during either positive or negative strand synthesis.

[0069] Example 2

[0070] This example illustrates plasmid construction of vectors, including plasmids comprising part of a CCMV RNA3 gene 3a open reading frame (ORF) plus all or part of a CCMV RNA3 3' UTR.

[0071] Wild type CCMV RNA3 has a 3a gene ORF and a coat protein (CP) ORF (figure 2). A cDNA copy is maintained in plasmid pCC3TP4. A *Not I* restriction site was introduced near the 3' end of the CP gene ORF in plasmid pCC3AG1 as well as the viral transgenes in transgenic plants 3-57 and Δ69 (Greene and Allison, *Science* 263: 1423-1425, 1994; Greene and Allison, *Virology* 225: 231-234, 1996). Transgenic plant 3-57 was transformed with the 3' 2/3 of the CP ORF and the full-length 3' UTR. Transgenic plant Δ69 was transformed with the same viral gene, but the 3' UTR bears a a 69-nucleotide deletion at the 3' end. The negative sense RNA-specific primer RA83 (5'-AAGTGGATCCCCTC TTGTGCGGCTGC-3' (SEQ ID NO: 1)) anneals at nucleotides 1519-1544, and was used for first strand cDNA synthesis and PCR. An additional primer RA84 (5'-ACTCCAAAGAGTTCTTCCG-3' (SEQ ID NO: 2)) anneals at nucleotides 2072-2090, and was used for PCR.

[0072] Example 3

[0073] This example illustrates synthesis of a complementary copy of a viral transgene during viral replication, as well as detection of synthesis of a complementary copy of a viral transgene during viral replication.

[0074] A study was undertaken to determine if infection of a transgenic plant with either a wild type brome mosaic bromovirus (BMV) or a CCMV leads to synthesis of a complementary copy of a transcript of a viral transgene.

[0075] Three sets of plant materials were used in the study: nontransgenic *Nicotiana benthamiana*, clonally propagated transgenic *N. benthamiana* strain 3-57 and clonally propagated transgenic *N. benthamiana* strain Δ69. Strain 3-57 comprises a 694 nucleotide CCMV transgene comprising 451 3' nucleotides of the viral coat gene and a complete 243 nucleotide CCMV 3' UTR that is naturally contiguous with the viral coat protein gene (Greene and Allison, *Science* 263: 1423-1425, 1994). Transgenic strain Δ69 is similar but except that the terminal 69 nucleotides of the 3' UTR are deleted. Transgenic transcripts comprising a fragment of the transgenic coat gene used in both transgenic strains were distinguishable from wild type viral transcripts comprising coat gene by the alteration of nucleotides near the 3' end of the coat gene to create a *Not I* restriction site in each transgene (Greene and Allison, *Virology* 225: 231-234, 1996). Northern blot analysis indicated that both strains express transcripts of the transgenes.

[0076] BMV and CCMV are both positive strand single-stranded RNA viruses, no DNA stage. Although these viruses share tripartite genomic organization, they show only limited sequence identity. Source plants for BMV and CCMV were inoculated with BMV and CCMV transcripts. Transgenic plants used in the experiments were inoculated with leaf tissue extracts from the BMV- or CCMV-infected plants.

[0077] To define a period when virus replication was most active throughout the plant following basal leaf inoculation, digoxygenin-labeled probes specific for the 3' UTR of the genomic RNAs of BMV and CCMV, (HE1 and RA518(+), respectively) were used in dot blot

assays to probe crude extracts derived from *N. benthamiana* plants inoculated with either BMV or CCMV. Hybridization indicated that 14 days post inoculation infections had spread to all leaves of 45-day old plants. However, attempts to detect minus strand copies of CCMV RNAs directly in total RNA extracted from non-transgenic plants in Northern blots were unsuccessful. Because of the possibility that an overwhelming amount of host and viral single strand RNA interfered with hybridization, single stranded RNA was removed from total RNA preparations by RNase treatment. Double-stranded RNA remaining after the RNase treatment was denatured and analyzed by Northern blot. In the resulting blots, minus strand CCMV RNA was detected by a ^{32}P-labeled RNA probe, (RA518(-)) which was able to recognize the complementary copy of the 3' UTR of CCMV RNA. Using this probe, minus-sense genomic CCMV RNAs were detected in 0.5 to 1.0 gram samples of CCMV-infected transgenic *N. benthamiana* plant tissue at 14 days post infection (dpi). Probes were shown to be capable of detecting as little as 10 picogram (pg) of denatured plasmid DNA comprising CCMV sequence.

[0078] To determine whether a complementary copy of the transgenes was synthesized during virus infection, transgenic lines 3-57 and Δ69 were inoculated with either BMV or CCMV. At 14 dpi, RNA was subjected to analysis by a reverse transcription-polymerase chain reaction (RT-PCR) method (figure 3). In this procedure, total RNA from two grams of leaf tissues was extracted, and treated with RNase-free DNase I to remove the plant genomic DNA, including the chromosomal copy of the viral genome. A minus-strand CCMV RNA3-specific primer, "RA83," comprising the sequence 5'-AAGTGGATCCCCTC TTGTGCGGCTGC-3' (SEQ ID NO: 1), which anneals to nucleotides 1519-1544 of the transgenes was used for first-strand cDNA synthesis. PCR amplification employed RA83 as well as "RA84," which comprises the sequence 5'-ACTCCAAAGAGTTCTTCCG-3' (SEQ ID NO: 2). RA84 anneals to

nucleotides 2072-2090 of the transgenes. The predicted size of an a fragment amplified by RT-PCR was 572 base pairs (bp). Analysis of RNA from plant tissue samples revealed that minus-strand RNA of the predicted size was synthesized in CCMV-infected plants of all strains tested, including 3-57, Δ69, and nontransgenic plants (figure 3). As shown in figure 3, lanes 7, 10 and 13, a minus-strand RNA was amplified in all the CCMV-infected 3-57, Δ69, and nontransgenic plants. A band of the predicted size was also present in the BMV-infected 3-57 transgenic plants (figure 3, lane 12). This minus-sense RNA was not observed in any mock-inoculated transgenic plants or in BMV-infected Δ69 or non-transgenic plants (figure 3, lanes 5, 6, 8, 9 and 11).

[0079] To determine if the 572 bp PCR products originated from transgene transcripts, PCR products were digested with *Not I*. Because only the transgene product but not the wild-type CCMV inoculum comprises a *Not I* restriction site, a change in electrophoretic mobility is expected only from digestion of cDNAs of the trangenes. As shown in figure 4, lane 3, RT-PCR product of pCC3AG1 mutant containing the *Not I* site was completely cleaved by the *Not I* restriction enzyme, but that of wild type CCMV was not cleaved (figure 4, lane 7), indicating that the minus-strand CCMV RNA amplified in CCMV-infected Δ69 transgenic plants was from the wild type CCMV. The RT-PCR product of CCMV-infected transgenic 3-57 plants was partially cleaved by *Not I* (figure 4, lane 11), indicating that for CCMV-infected transgenic 3-57 plants, the minus-sense CCMV RNA3 which was amplified by RT-PCR was of two origins: both viral and transgene. For BMV-infected 3-57 transgenic plants, the RT-PCR product was completely cleaved by *Not I*. No PCR fragment was present in the BMV-inoculated Δ69 plants (figure 3, lane 9). The data indicate that a full-length complementary copy of a transcript of a viral transgene is synthesized only when the 3' UTR of the viral transgene is intact. Thus the RT-PCR-amplified fragment was derived solely from a complementary copy of a viral transgene

transcript. These data indicate that BMV recognized a replicase recognition and minus-strand initiation site on the CCMV transgene and synthesized a complementary copy. Although F_1 seedlings of 3-57 plants were used in these experiments, similar reults were obtained when F_0 plant cuttings were used in repeated experiments.

[0080] These data demonstrate that the replication complex of either BMV or CCMV will recognize and synthesize a complementary copy of a CCMV transgene that contains a complete 3' UTR. Together with the findings of Teycheney et al. (*J. Gen. Virol.* 81: 1121-1126, 2000), the data demonstrate in several plant viral systems that inclusion of the replication complex binding site in a transgenic construct may lead to the synthesis of the transgene's complement.

[0081] Example 4

[0082] This example illustrates a prophetic example of expression of a transgene. In this prophetic example, a DNA transgene could comprise a CaMV 35S promoter operatively linked to a complementary copy of a sequence encoding barnase (a cell toxin; Leuchtenberger et al., *Nucleic Acid Res.* 29: E76, 2001), a complementary copy of an encephalomyocarditis virus IRES, and a 3' UTR of a Cowpea chlorotic mottle virus. Cells of a *Nicotiana benthamiana* plant harboring this transgene are expected to express an RNA comprising complementary copy of the sequence encoding barnase, a complementary copy of the encephalomyocarditis virus IRES, and the 3' UTR of cowpea chlorotic mottle virus. No barnase is expected to be detectable in any plant tissue prior to application of a stimulus. Upon infection of the plant with a virus such as a Cowpea chlorotic mottle virus or a Brome mosaic virus, a complementary copy of the recombinant RNA is expected to be produced. Because a complementary copy of the recombinant RNA is expected to comprise both the encephalomycocarditis virus IRES and a

"sense" copy of the sequence encoding the barnase, it is expected that an infected cell will synthesize the barnase. Because cell death is expected to result from barnase expression, the virus is expected to be unable to replicate in infected cells, and the viral infection is expected to be unable to spread from cell to cell.

[0083] Example 5

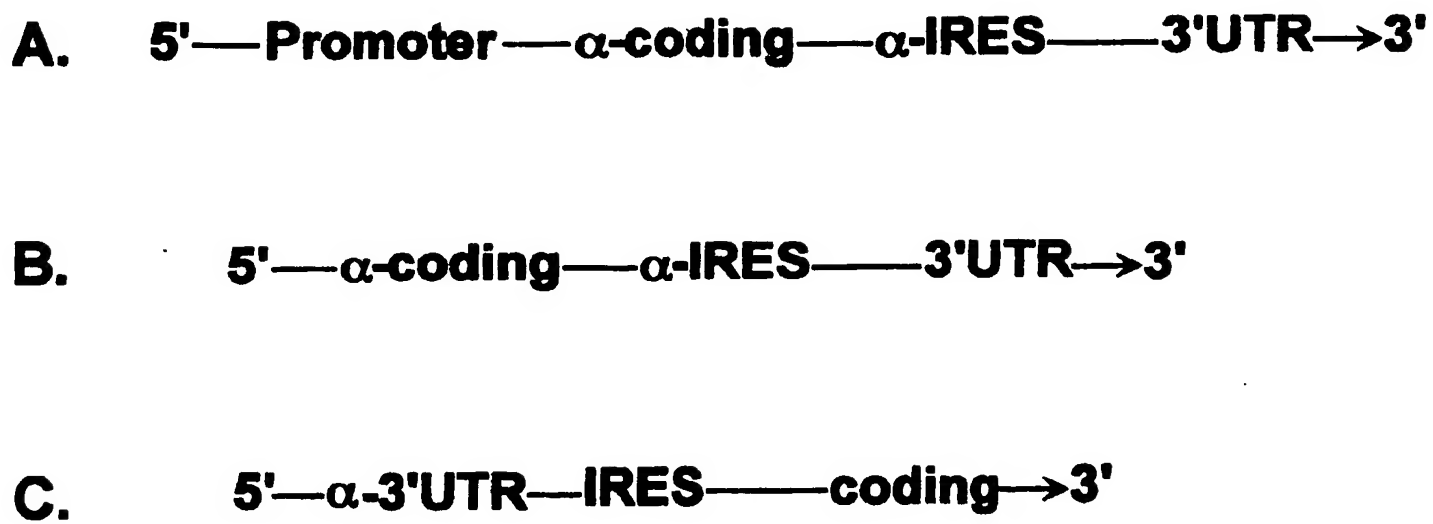
[0084] This example illustrates a prophetic example of expression of a transgene. In this prophetic example, a DNA transgene could comprise a CaMV 35S promoter operatively linked to a complementary copy of a sequence encoding preproinsulin, a complementary copy of an encephalomyocarditis virus IRES, and a 3' UTR of a Cowpea chlorotic mottle virus. Cells of a *Nicotiana benthamiana* plant harboring this transgene are expected to express an RNA comprising a complementary copy of the sequence encoding preproinsulin, a complementary copy of the encephalomyocarditis virus IRES, and the 3' UTR of cowpea chlorotic mottle virus. No preproinsulin is expected to be detectable in any plant tissue prior to application of a stimulus. Upon infection of the plant with a virus such as a Cowpea chlorotic mottle virus or a Brome mosaic virus, a complementary copy of the recombinant RNA is expected to be produced. Because a complementary copy of the recombinant RNA is expected to comprise both the encephalomycocarditis virus IRES and a "sense" copy of the sequence encoding the preproinsulin, it is expected that an infected cell will synthesize the preproinsulin, and as the infection spreads throughout the plant, additional cells are expected to synthesize preproinsulin.

[0085] As various changes could be made in the above methods and compositions without departing from the scope of the invention, it is intended that all matter contained in the above description be interpreted as illustrative and not in a limiting sense.

[0086] All references cited in this specification are hereby incorporated by reference in their entirety. The discussion of the references herein is intended merely to summarize the assertions made by their authors and no admission is made that any reference constitutes prior art relevant to patentability. Applicant reserves the right to challenge the accuracy and pertinency of the cited references.

ABSTRACT

A system for expression of a heterologous polypeptide in a transgenic host cell is disclosed. The system is based upon a transgene comprising a eukaryotic promoter operably linked to a DNA sequence comprising, in the 5' to 3' direction, a DNA sequence complementary to a sequence encoding a heterologous polypeptide, a DNA sequence complementary to an internal ribosome entry site, and a DNA sequence corresponding to a 3' untranslated region of a positive strand single-stranded RNA virus. Following introduction of a stimulus, the host cell synthesizes an RNA molecule complementary to a recombinant RNA encoded by the transgene. The stimulus can be a positive strand single-stranded RNA virus or a nucleic acid thereof. Because the complement of the recombinant RNA comprises an internal ribosome entry site and a sequence encoding a heterologous polypeptide, the host cell can synthesize the heterologous polypeptide.


A. 5'—Promoter—α-coding—α-IRES——3'UTR→3'
B. 5'—α-coding—α-IRES——3'UTR→3'
C. 5'—α-3'UTR—IRES——coding→3'

FI URE 1

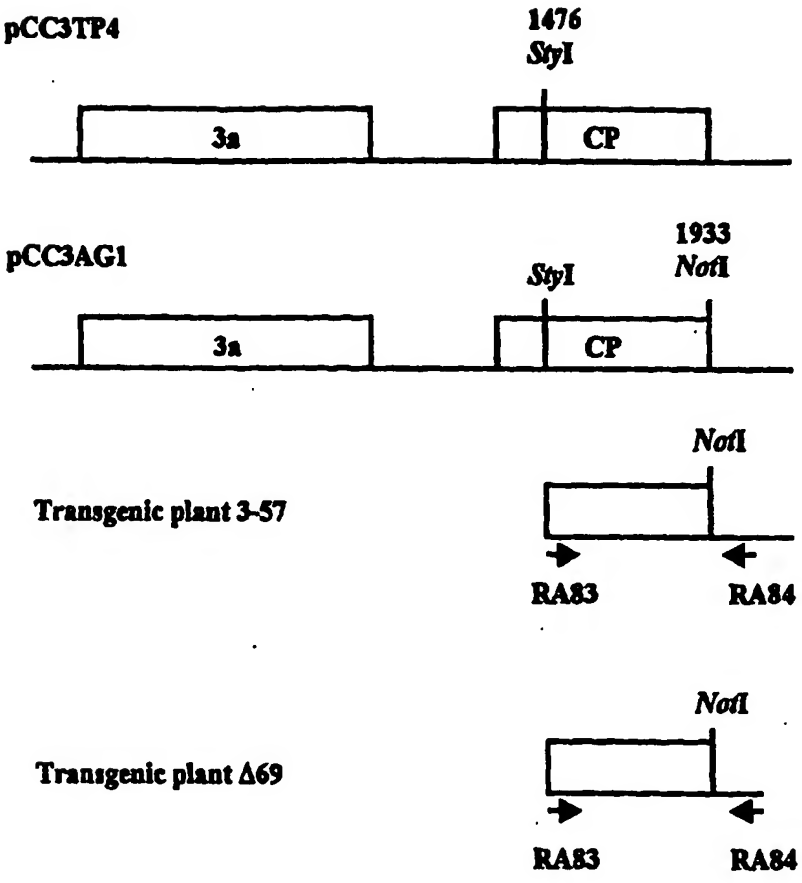


Figure 1. Plasmid construction. Wild type CCMV RNA3 has a 3a gene ORF and a coat protein (CP) ORF, and it is maintained in plasmid pCC3TP4. A Not I restriction site was introduced near the 3' end of the CP gene ORF in plasmid pCC3AG1 as well as the viral transgenes in transgenic plants 3-57 and Δ69 (Greene and Allison, 1994;1996). Transgenic plant 3-57 was transformed with the 3' 2/3 of the CP ORF and the full-length 3' UTR. Transgenic plant Δ69 was transformed with the same viral gene, but the 3' UTR bears a 69-nucleotide deletion at the 3' end. The negative sense RNA specific primer RA83 (5' AAGTGGATCCCCTCTTGTGCGGCTGC 3') anneals at nucleotides 1519-1544, and was used for first strand cDNA synthesis. An additional primer RA84 (5' ACTCCAAAGAGTTCTTCCG 3') anneals at nucleotides 2072-2090, and was used for PCR.

FI URE 2

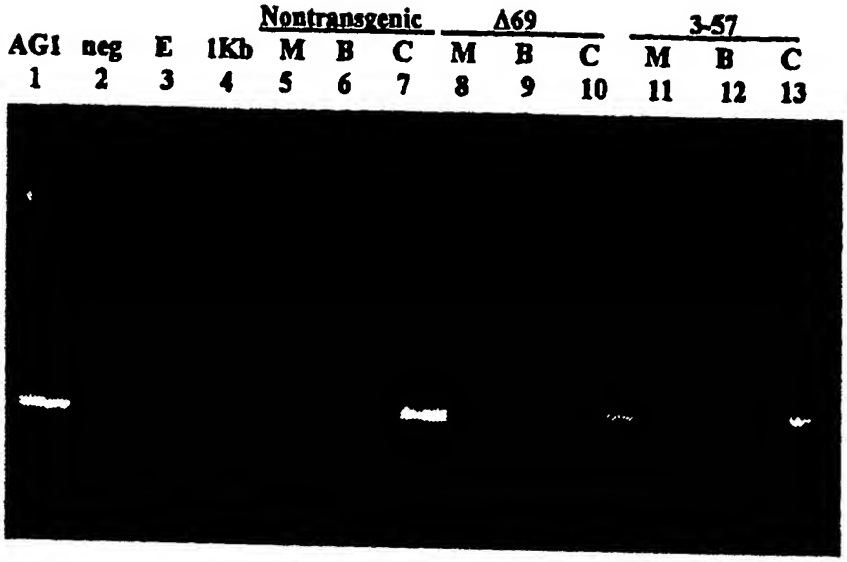


Figure 4. Agarose gel electrophoresis showing RT-PCR amplified negative-sense CCMV RNA3. Total RNA from virus infected or mock-inoculated transgenic and nontransgenic *N. benthamiana* plant tissue was treated with RNase free DNase I to remove the genomic DNA. A negative-sense CCMV RNA3 specific primer RA83 (5' AAGTGATCCCCTCTTGTGCGGCTGC 3'), which anneals at nucleotide 1519-1544, was used for first-strand cDNA synthesis. An additional primer RA84 (5' ACTCCAAAGAGTTCTTCCG 3'), which anneals at nucleotide 2072-2090, was used in PCR. Lanes 5-7, 8-10, and 11-13 were from nontransgenic, transgenic Δ69, and 3-57 plants, respectively. Samples in lanes 5, 8, and 11 were mock-inoculated (M); lanes 6, 9, and 12 were inoculated with BMV (B); lanes 7, 10, and 13 were inoculated with CCMV (C). Lane 1 contains PCR product using 0.1 μg pCC3AG1 plasmid DNA. Lane 2 is the negative control of PCR where water was added to the PCR mixture. Lane 3 is empty (E). Lane 4 contains 1 Kb size marker (GibcoBRL).

FIGURE 3

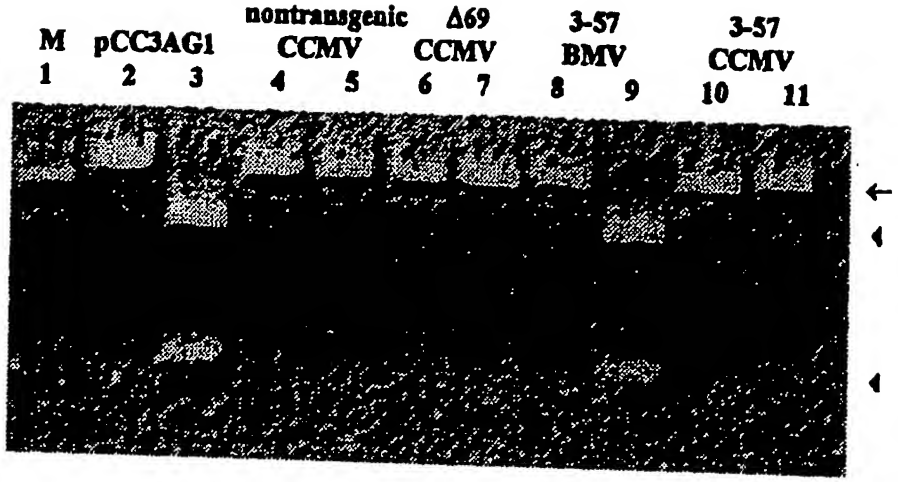


Figure 5. Agarose gel showing *Not*I treated RT-PCR products amplified from total RNA extracted from virus infected plant tissue. Lane 1 contains the 1 Kb size marker (GibcoBRL). Lanes 2-3 contain PCR products amplified from pCC3AG1. RT-PCR amplified products were from CCMV infected nontransgenic *N. benthamiana* plants (lanes 4-5); CCMV infected Δ69 plants (lanes 6-7); BMV infected 3-57 plants (lanes 8-9); CCMV infected 3-57 plants (lanes 10-11). RT-PCR products in lanes 2, 4, 6, 8, and 10 were not treated with *Not*I restriction enzyme, whereas those in lanes 3, 5, 7, 9, and 11 were treated with *Not*I. Arrow "←" indicates undigested fragments. Arrow "◀" indicates digested fragments. Note the small digested bands in lane 11.

FI URE 4

a. Double Strand DNA — 5'- Promoter - Antisense Gene - Antisense IRES - Viral 3' UTR - 3'
3'- Promoter - Antisense Gene - Antisense IRES - Viral 3' UTR - 5'

b. RNA Polymerase II Transcript — 5' – Antisense Gene - Antisense IRES - Viral 3' UTR - 3'

c. Product of Viral Replication Complex — 5' – Viral 3' UTR - Functional IRES - Functional Gene - 3'

Generalized arrangement of components *in planta*. (a) Double Strand DNA Transgene Complex is shown at the top. The plant RNA polymerase II recognizes the transcriptional promoter and produces the RNA transcript shown in paanel (b) The Pol II transcript is transported to the cytoplasm where it awaits the virus that recognizes its 3' UTR as a replication initiation site. Upon introduction of the appropriate RNA virus, the viral replication complex recognizes its 3' UTR and makes a complementary RNA copy (c) of Pol II transcript. The Functional IRES enables the entry of a ribosome and the translation of the transgene that is now in the sense orientation.

FIGURE 5

a.

DNA 5' - Promoter *YYYCAT GGAATT Viral 3' UTR – 3'
3' – Promoter *XXXGTA CCTTAA Viral 3'UTR – 5'

b.

RNA 5'- *YYYCAU GGAAUU Viral 3' UTR – 3'

c.

RNA 5' - Viral 3' UTR AAUUCC AUGXXX* - 3'

FIGURE 9

Detailed arrangement of components *in planta*. Panel (a) shows the antisense relationship of the gene and IRES to the promoter and viral 3' UTR (untranslated region). Upside down nucleotides indicate antisense orientation. In panel (b) the transcript of RNA polymerase is shown with the gene and IRES in the upside down antisense orientation. The replication complex of the appropriate virus will recognize its 3' UTR and convert the polymerase II transcript to its complement shown in panel (c). This final conversion results in both the IRES and gene in a translatable orientation. AATTCC – IRES, ATG – initiation codon, XXX – any codon, YYY – complement of codon, * - stop codon

INVENTOR INFORMATION

Inventor One Given Name:: Richard F
Family Name:: Allison
City:: East Lansing
State or Province:: Michigan
Country:: United States
Postal or Zip Code:: 48824
City of Residence:: East Lansing
State or Province of Residence:: Michigan
Country of Residence:: United States
Citizenship Country:: United States

CORRESPONDENCE INFORMATION

Correspondence Customer Number:: 27572
Fax One:: 248-641-0270

APPLICATION INFORMATION

Title Line One:: EXPRESSION OF A RECOMBINANT TRANSGENE
Total Drawing Sheets:: 6
Formal Drawings?:: No
Application Type:: Provisional
Docket Number:: 6550-000072
Secrecy Order in Parent Appl.?:: No

Source:: PrintEFS Version 1.0.1

Please type a plus sign (+) inside this box → [+]

PTO/SB/16 (10-01)
Approved for use through 10/31/2002. OMB 0651-0032
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

PTO 10447 U.S. 60/485073 07/03/03

Express Mail Label No. EL 991953750 US

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Richard F.	Allison	East Lansing, Michigan

☐ *Additional inventors are being named on the _____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (280 characters max)

EXPRESSION OF A RECOMBINANT TRANSGENE

CORRESPONDENCE ADDRESS

Direct all correspondence to:

☒ Customer Number 27572 →

OR *Type Customer Number here*

☐ Firm *or* Individual Name	Harness, Dickey & Pierce, P.L.C.				
Address	P.O. Box 828				
Address					
City	Bloomfield Hills	State	MI	ZIP	48098
Country	USA	Telephone	248-641-1600	Fax	248-641-0270

ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* 114 ☐ CD(s), Number

☒ Drawing(s) *Number of Sheets* 6 ☐ Other (specify)

☒ Application Data Sheet. See 37 CFR 1.76 ☒ Specification Filed in English

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

☒ Applicant claims small entity status. See 37 CFR 1.27.

☒ A check or money order is enclosed to cover the filing fees

☒ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 08-0750

☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT ($): 80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____.

Respectfully submitted,

SIGNATURE Saul Zackson Date July 3, 2003

TYPED or PRINTED NAME Saul L. Zackson REGISTRATION NO. *(if appropriate)* 52,391

TELEPHONE 314-259-7500 Docket Number: 6550-000072

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

EL 991953750 US

PTO/SB/17 (01-03)
Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

FEE TRANSMITTAL for FY 2003

Patent fees are subject to annual revision.

☒ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 80.00

Complete If Known	
Application Number	Unknown
Filing Date	July 3, 2003
First Named Inventor	Richard Allison
Examiner Name	Unknown
Group / Art Unit	Unknown
Attorney Docket No.	6550-000072

METHOD OF PAYMENT (check all that apply)

☒ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

☒ Deposit Account:

Deposit Account Number: 08-0750

Deposit Account Name: Hamess, Dickey & Pierce, P.L.C.

The Commissioner is authorized to: *(check all that apply)*
☐ Charge fee(s) indicated below ☒ Credit any overpayments
☒ Charge any additional fee(s) during the pendency of this application
☐ Charge fee(s) indicated below, **except for the filing fee** to the above-identified deposit account.

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	750	2001	375	Utility filing fee	
1002	330	2002	165	Design filing fee	
1003	520	2003	260	Plant filing fee	
1004	750	2004	375	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80.00

SUBTOTAL (1) ($) 80.00

2. EXTRA CLAIM FEES

			Extra Claims		Fee from below		Fee Paid
Total Claims		- ** =	0	X		=	0
Independent Claims		- ** =	0	X		=	0
Multiple Dependent				X		=	0

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	84	2201	42	Independent claims in excess of 3
1203	280	2203	140	Multiple dependent claim, if not paid
1204	84	2204	42	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 0

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet.	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	410	2252	205	Extension for reply within second month	
1253	930	2253	465	Extension for reply within third month	
1254	1,450	2254	725	Extension for reply within fourth month	
1255	1,970	2255	985	Extension for reply within fifth month	
1401	320	2401	160	Notice of Appeal	
1402	320	2402	160	Filing a brief in support of an appeal	
1403	280	2403	140	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive – unavoidable	
1453	1,300	2453	650	Petition to revive – unintentional	
1501	1,300	2501	650	Utility issue fee (or reissue)	
1502	470	2502	235	Design issue fee	
1503	630	2503	315	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17 (q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	750	2809	375	Filing a submission after final rejection (37 CFR § 1.129(a))	
1810	750	2810	375	For each additional invention to be examined (37 CFR § 1.129(b))	
1801	750	2801	375	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	
Other fee (specify) ____					

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($) 0

SUBMITTED BY

				Complete *(if applicable)*	
Name (Print/Type)	Saul L. Zackson	*Registration No. Attorney/Agent)*	52,391	*Telephone*	314-259-7500
Signature	Saul L. Zack			*Date*	July 3, 2003

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

EL 991953750 US

Please type a plus sign (+) inside this box → [+]

HDP/SB/21 based on PTO/SB/21 (08-00)

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	Unknown
Filing Date	July 3, 2003
First Named Inv ntor	Richard Allison
Group Art Unit	Unknown
Examiner Name	Unknown
Attorney Docket Number	6550-000072

Total Number of Pages in This Submission

ENCLOSURES *(check all that apply)*

- ☒ Fee Transmittal Form
  - ☒ Fee Attached
- ☐ Amendment / Response
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Response to Missing Parts/ Incomplete Application
  - ☐ Response to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Assignment Papers *(for an Application)*
- ☒ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☐ Power of Attorney, Revocation Change of Correspondence Address
- ☐ Terminal Disclaimer
- ☐ Request for Refund
- ☐ CD, Number of CD(s) ____
- ☐ After Allowance Communication to Group
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☐ Appeal Communication to Group *(Appeal Notice, Brief, Reply Brief)*
- ☐ Proprietary Information
- ☐ Status Letter
- ☒ Other Enclosure(s) *(please identify below)*:
  - **Provisional Application**
  - **Provisional Application for Patent Cover Sheet (1 page)**
  - **Check in the amount of $80.00**
  - **Patent Application Bibliographic Data**
  - **Return Receipt Postcard**

Remarks: **The Commissioner is hereby authorized to charge any additional fees that may be required under 37 CFR 1.16 or 1.17 to Deposit Account No. 08-0750. A duplicate copy of this sheet is enclosed.**

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm *or* Individual name	Harness, Dickey & Pierce, P.L.C.	Attorney Name Saul Zackson	Reg. No. 52,391
Signature	Saul L. Zackson		
Date	July 3, 2003		

CERTIFICATE OF MAILING/TRANSMISSION

I hereby certify that this correspondence is being deposited with the United States Postal Service as Express Mail Label No.: EL 991953750 US in an envelope addressed to: Mail Stop Provisional Patent Application, Commissioner for Patents, Office, P.O. Box 1450, Alexandria, VA 22313-1450 on this date: July 3, 2003

Typed or printed name	Saul L. Zackson		
Signature	Saul L. Zackson	Date	July 3, 2003

EL 991953750 US

I claim:

1. A method of conferring disease resistance to a transgenic plant, the method comprising

a) providing a transgenic plant comprising a recombinant DNA molecule comprising a promoter operably linked to a DNA sequence comprising, in the 5' to 3' direction,

i) a sequence complementary to a coding sequence for a heterologous polypeptide capable of conferring disease resistance;

ii) a sequence complementary to an internal ribosome entry site;

iii) a 3' UTR of a first positive strand single-stranded RNA virus; and

b) growing the transgenic plant,

whereby resistance is conferred to infection from a second positive strand single-stranded RNA virus.

2. The method of conferring disease resistance to a transgenic plant of claim 1, wherein the promoter is selected from the group consisting of a constitutive promoter and an inducible promoter.

3. The method of conferring disease resistance to a transgenic plant of claim 2, wherein the promoter is a constitutive promoter.

4. The method of conferring disease resistance to a transgenic plant of claim 3, wherein the constitutive promoter is a eukaryotic constitutive promoter selected from the group consisting of a cauliflower mosaic virus 35S promoter, a blueberry red ringspot virus promoter, a ubiquitin gene promoter, an actin gene promoter, an NeIF-4A10 promoter, a maize Adh1-based

pEmu promoter, a barley leaf thionin BTH6 promoter, a cassava vein mosaic virus promoter, a sugarcane bacilliform badnavirus promoter and a histone gene promoter.

5. The method of conferring disease resistance to a transgenic plant of claim 4, wherein the eukaryotic constitutive promoter is a cauliflower mosaic virus 35S promoter.

6. The method of conferring disease resistance to a transgenic plant of claim 5, wherein the cauliflower mosaic virus 35S promoter comprises the sequence:

AGATTAGCCTTTTCAATTTCAGAAAGAATGCTAACCCACAGATGGTTAGAGAGGCTT
ACGCAGCAGGTCTCATCAAGACGATCTACCCGAGCAATAATCTCCAGGAAATCAAA
TACCTTCCCAAGAAGGTTAAAGATGCAGTCAAAAGATTCAGGACTAACTGCATCAA
GAACACAGAGAAAGATATATTTCTCAAGATCAGAAGTACTATTCCAGTATGGACGA
TTCAAGGCTTGCTTCACAAACCAAGGCAAGTAATAGAGATTGGAGTCTCTAAAAAG
GTAGTTCCCACTGAATCAAAGGCCATGGAGTCAAAGATTCAAATAGAGGACCTAAC
AGAACTCGCCGTAAAGACTGGCGAACAGTTCATACAGAGTCTCTTACGACTCAATG
ACAAGAAGAAAATCTTCGTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAA
AATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAG
GGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTG
AAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAA
GGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCA
CGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGAT
TGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAA
GACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGAACACG (SEQ ID NO: 3).

7. The method of conferring disease resistance in a transgenic cell of claim 1, wherein the coding sequence for a heterologous polypeptide encodes a polypeptide selected from the group consisting of a cell toxin and a viral polypeptide.

8. The method of conferring disease resistance in a transgenic cell of claim 7, wherein the viral polypeptide is a viral coat protein polypeptide.

9. The method of conferring disease resistance to a transgenic plant of claim 1, wherein the sequence complementary to an IRES is a sequence complementary to an IRES selected from the group consisting of a picornavirus IRES, a foot-and-mouth disease virus IRES, an encephalomyocarditis virus IRES, a hepatitis A virus IRES, a hepatitis C virus IRES, a human rhinovirus IRES, a poliovirus IRES, a swine vesicular disease virus IRES, a turnip mosaic potyvirus IRES, a human fibroblast growth factor 2 mRNA IRES, a pestivirus IRES, a Leishmania RNA virus IRES, a Moloney murine leukemia virus IRES a human rhinovirus 14 IRES, an aphthovirus IRES, a human immunoglobulin heavy chain binding protein mRNA IRES, a *Drosophila* Antennapedia mRNA IRES, a human fibroblast growth factor 2 mRNA IRES, a hepatitis G virus IRES, a tobamovirus IRES, a vascular endothelial growth factor mRNA IRES, a Coxsackie B group virus IRES, a c-myc protooncogene mRNA IRES, a human MYT2 mRNA IRES, a human parechovirus type 1 virus IRES, a human parechovirus type 2 virus IRES, a eukaryotic initiation factor 4GI mRNA IRES, a Plautia stali intestine virus IRES, a Theiler's murine encephalomyelitis virus IRES, a bovine enterovirus IRES, a connexin 43 mRNA IRES, a homeodomain protein Gtx mRNA IRES, an AML1 transcription factor mRNA IRES, an NF-kappa B repressing factor mRNA IRES, an X-linked inhibitor of apoptosis mRNA IRES, a cricket paralysis virus RNA IRES, a p58(PITSLRE) protein kinase mRNA IRES, an ornithine decarboxylase mRNA IRES, a connexin-32 mRNA IRES, a bovine viral diarrhea virus

IRES, an insulin-like growth factor I receptor mRNA IRES, a human immunodeficiency virus type 1 gag gene IRES, a classical swine fever virus IRES, a Kaposi's sarcoma-associated herpes virus IRES, a short IRES selected from a library of random oligonucleotides, a Jembrana disease virus IRES, an apoptotic protease-activating factor 1 mRNA IRES, a Rhopalosiphum padi virus IRES, a cationic amino acid transporter mRNA IRES, a human insulin-like growth factor II leader 2 mRNA IRES, a giardiavirus IRES, a Smad5 mRNA IRES, a porcine teschovirus-1 talfan IRES, a *Drosophila* Hairless mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2 mRNA IRES, an Epstein-Barr virus IRES, a hibiscus chlorotic ringspot virus IRES, a rat pituitary vasopressin V1b receptor mRNA IRES, and a human hsp70 mRNA IRES.

10. The method of conferring disease resistance to a transgenic plant of claim 9, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to a picornavirus internal ribosome entry site.

11. The method of conferring disease resistance to a transgenic plant of claim 10, wherein the sequence complementary to a picornavirus internal ribosome entry site comprises the sequence:

TTATCATCGTGTTTTTCAAAGGAAAACCACGTCCCCGTGGTTCGGGGGGCCTAGACG
TTTTTTTAACCTCGACTAAACACATGTAAAGCATGTGCACCGAGGCCCCAGATCAGA
TCCCATACAATGGGGTACCTTCTGGGCATCCTTCAGCCCCTTGTTGAATACGCTTGA
GGAGAGCCATTTGACTCTTTCCACAACTATCCAACTCACAACGTGGCACTGGGGTTG
TGCCGCCTTTGCAGGTGTATCTTATACACGTGGCTTTTGGCCGCAGAGGCACCTGTC
GCCAGGTGGGGGGTTCCGCTGCCTGCAAAGGGTCGCTACAGACGTTGTTTGTCTTCA
AGAAGCTTCCAGAGGAACTGCTTCCTTCACGACATTCAACAGACCTTGCATTCCTTT
GGCGAGAGGGGAAAGACCCCTAGGAATGCTCGTCAAGAAGACAGGGCCAGGTTTCC

GGGCCCTCACATTGCCAAAAGACGGCAATATGGTGGAAAATCACATATAGACAAAC
GCACACCGGCCTTATTCCAAGCGGCTTCGGCCAGTAACGTTAGGGGGGGGGGAGGG
AGAGGGGCGGAATT (SEQ ID NO: 6).

12. The method of conferring disease resistance to a transgenic plant of claim 1, wherein the 3' UTR of a first positive strand single-stranded RNA virus is a 3' UTR of a first positive strand single-stranded RNA virus having no DNA stage.

13. The method of conferring disease resistance to a transgenic plant of claim 12, wherein the 3' UTR of a first positive strand single-stranded RNA virus having no DNA stage is a 3' UTR of a first bromovirus.

14. The method of conferring disease resistance to a transgenic plant of claim 13, wherein the 3' UTR of a first bromovirus is a 3' UTR of a first Cowpea chlorotic mottle virus.

15. The method of conferring disease resistance to a transgenic plant of claim 14, wherein a DNA copy of the 3' UTR of a first Cowpea chlorotic mottle virus comprises the sequence:

AGTGCCCGCTGAAGAGCGTTACACTAGTGTGGCCTACTTGAAGGCTAGTTATAACCG
TTTCTTTAAACGGTAATCGTTGTTGAAACGTCTTCCTTTTACAAGAGGATTGAGCTGC
CCTTGGGTTTTACTCCTTGAACCCTTCGGAAGAACTCTTTGGAGTTCGTACCAGTACC
TCACATAGTGAGGTAATAAGACTGGTGGGCAGCGCCTAGTCGAAAGACTAGGTGAT
CTCTAAGGAGACC (SEQ ID NO: 8).

16. The method of conferring disease resistance to a transgenic plant of claim 1, further comprising a sequence complementary to an intron.

17. The method of conferring disease resistance to a transgenic plant of claim 1, further comprising a transcription termination signal.

18. The method of conferring disease resistance to a transgenic plant of claim 1, wherein the plant is a dicotyledonous plant.

19. The method of conferring disease resistance to a transgenic plant of claim 19, wherein the dicotyledonous plant is a *Nicotiana* plant.

20. The method of conferring disease resistance to a transgenic plant of claim 20, wherein the *Nicotiana* plant is a *Nicotiana benthamiana* plant.

21. The method of conferring disease resistance to a transgenic plant of claim 1, wherein the second positive strand single-stranded RNA virus is a positive strand single-stranded RNA virus having no DNA stage.

22. The method of conferring disease resistance to a transgenic plant of claim 21, wherein the second positive strand single-stranded RNA virus having no DNA stage is selected from the group consisting of a positive strand single-stranded RNA plant virus having no DNA stage and a positive single-stranded RNA animal virus having no DNA stage.

23. The method of conferring disease resistance to a transgenic plant of claim 22, wherein the second positive strand single-stranded RNA plant virus having no DNA stage is selected from the group consisting of a second Bromovirus, a Tobacco etch virus, a Tobacco vein mottle virus, and a Pepper mottle virus.

24. The method of conferring disease resistance to a transgenic plant of claim 23, wherein the second Bromovirus is selected from a second Cowpea chlorotic mottle virus and a second Brome mosaic virus.

25. The method of conferring disease resistance to a transgenic plant of claim 23, wherein the second Bromovirus is a second Cowpea chlorotic mottle virus.

26. The method of conferring disease resistance to a transgenic plant of claim 1, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 50:1.

27. The method of conferring disease resistance to a transgenic plant of claim 26, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 100:1.

28. The method of conferring disease resistance to a transgenic plant of claim 27, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 1000:1.

29. The method of conferring disease resistance to a transgenic plant of claim 28, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 10,000:1.

30. A method of producing a heterologous polypeptide in a transgenic plant, the method comprising:

a) providing a transgenic plant comprising a recombinant DNA molecule comprising a promoter operably linked to a DNA sequence comprising, in the 5' to 3' direction,

i) a sequence complementary to a coding sequence for a heterologous polypeptide;

ii) a sequence complementary to an internal ribosome entry site;

iii) a 3' UTR of a first positive strand single-stranded RNA virus;

b) growing the transgenic plant; and

c) providing a stimulus to the transgenic plant for synthesis of an RNA complementary to an RNA transcript of the recombinant DNA.

31. The method of producing a heterologous polypeptide in a transgenic plant of claim 30, wherein the promoter is a selected from the group consisting of a constitutive promoter and an inducible promoter.

32. The method of producing a heterologous polypeptide in a transgenic plant of claim 31, wherein the promoter is a constitutive promoter.

33. The method of producing a heterologous polypeptide in a transgenic plant of claim 32, wherein the constitutive promoter is a eukaryotic constitutive promoter selected from the group consisting of a cauliflower mosaic virus 35S promoter, a blueberry red ringspot virus promoter, a ubiquitin gene promoter, an actin gene promoter, an NeIF-4A10 promoter, a maize Adh1-based pEmu promoter, a barley leaf thionin BTH6 promoter, a cassava vein mosaic virus promoter, a sugarcane bacilliform badnavirus promoter and a histone gene promoter.

34. The method of producing a heterologous polypeptide in a transgenic plant of claim 33, wherein the eukaryotic constitutive promoter is a cauliflower mosaic virus 35S promoter.

35. The recombinant DNA molecule of claim 34, wherein the cauliflower mosaic virus 35S promoter comprises the sequence:

AGATTAGCCTTTTCAATTTCAGAAAGAATGCTAACCCACAGATGGTTAGAGAGGCTT
ACGCAGCAGGTCTCATCAAGACGATCTACCCGAGCAATAATCTCCAGGAAATCAAA
TACCTTCCCAAGAAGGTTAAAGATGCAGTCAAAAGATTCAGGACTAACTGCATCAA
GAACACAGAGAAAGATATATTTCTCAAGATCAGAAGTACTATTCCAGTATGGACGA
TTCAAGGCTTGCTTCACAAACCAAGGCAAGTAATAGAGATTGGAGTCTCTAAAAAG

GTAGTTCCCACTGAATCAAAGGCCATGGAGTCAAAGATTCAAATAGAGGACCTAAC

AGAACTCGCCGTAAAGACTGGCGAACAGTTCATACAGAGTCTCTTACGACTCAATG

ACAAGAAGAAAATCTTCGTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAA

AATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAG

GGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTG

AAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAA

GGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCA

CGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGAT

TGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAA

GACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGAACACG (SEQ ID NO: 3).

36. The method of producing a heterologous polypeptide in a transgenic cell of claim 30, wherein the coding sequence for a heterologous polypeptide encodes a polypeptide selected from the group consisting of a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

37. The method of producing a heterologous polypeptide in a transgenic plant of claim 30, wherein the sequence complementary to an IRES is a sequence complementary to an IRES selected from the group consisting of a picornavirus IRES, a foot-and-mouth disease virus IRES, an encephalomyocarditis virus IRES, a hepatitis A virus IRES, a hepatitis C virus IRES, a human rhinovirus IRES, a poliovirus IRES, a swine vesicular disease virus IRES, a turnip mosaic potyvirus IRES, a human fibroblast growth factor 2 mRNA IRES, a pestivirus IRES, a Leishmania RNA virus IRES, a Moloney murine leukemia virus IRES a human rhinovirus 14 IRES, an aphthovirus IRES, a human immunoglobulin heavy chain binding protein mRNA IRES, a *Drosophila* Antennapedia mRNA IRES, a human fibroblast growth factor 2 mRNA

IRES, a hepatitis G virus IRES, a tobamovirus IRES, a vascular endothelial growth factor mRNA IRES, a Coxsackie B group virus IRES, a c-myc protooncogene mRNA IRES, a human MYT2 mRNA IRES, a human parechovirus type 1 virus IRES, a human parechovirus type 2 virus IRES, a eukaryotic initiation factor 4GI mRNA IRES, a Plautia stali intestine virus IRES, a Theiler's murine encephalomyelitis virus IRES, a bovine enterovirus IRES, a connexin 43 mRNA IRES, a homeodomain protein Gtx mRNA IRES, an AML1 transcription factor mRNA IRES, an NF-kappa B repressing factor mRNA IRES, an X-linked inhibitor of apoptosis mRNA IRES, a cricket paralysis virus RNA IRES, a p58(PITSLRE) protein kinase mRNA IRES, an ornithine decarboxylase mRNA IRES, a connexin-32 mRNA IRES, a bovine viral diarrhea virus IRES, an insulin-like growth factor I receptor mRNA IRES, a human immunodeficiency virus type 1 gag gene IRES, a classical swine fever virus IRES, a Kaposi's sarcoma-associated herpes virus IRES, a short IRES selected from a library of random oligonucleotides, a Jembrana disease virus IRES, an apoptotic protease-activating factor 1 mRNA IRES, a Rhopalosiphum padi virus IRES, a cationic amino acid transporter mRNA IRES, a human insulin-like growth factor II leader 2 mRNA IRES, a giardiavirus IRES, a Smad5 mRNA IRES, a porcine teschovirus-1 talfan IRES, a *Drosophila* Hairless mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2 mRNA IRES, an Epstein-Barr virus IRES, a hibiscus chlorotic ringspot virus IRES, a rat pituitary vasopressin V1b receptor mRNA IRES, and a human hsp70 mRNA IRES.

38. The method of producing a heterologous polypeptide in a transgenic plant of claim 37, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to a picornavirus internal ribosome entry site.

39. The method of producing a heterologous polypeptide in a transgenic plant of claim 38, wherein the sequence complementary to a picornavirus internal ribosome entry site

comprises the sequence:

TTATCATCGTGTTTTTCAAAGGAAAACCACGTCCCCGTGGTTCGGGGGGCCTAGACG
TTTTTTTAACCTCGACTAAACACATGTAAAGCATGTGCACCGAGGCCCCAGATCAGA
TCCCATACAATGGGGTACCTTCTGGGCATCCTTCAGCCCCTTGTTGAATACGCTTGA
GGAGAGCCATTTGACTCTTTCCACAACTATCCAACTCACAACGTGGCACTGGGGTTG
TGCCGCCTTTGCAGGTGTATCTTATACACGTGGCTTTTGGCCGCAGAGGCACCTGTC
GCCAGGTGGGGGGTTCCGCTGCCTGCAAAGGGTCGCTACAGACGTTGTTTGTCTTCA
AGAAGCTTCCAGAGGAACTGCTTCCTTCACGACATTCAACAGACCTTGCATTCCTTT
GGCGAGAGGGGAAAGACCCCTAGGAATGCTCGTCAAGAAGACAGGGCCAGGTTTCC
GGGCCCTCACATTGCCAAAAGACGGCAATATGGTGGAAAATCACATATAGACAAAC
GCACACCGGCCTTATTCCAAGCGGCTTCGGCCAGTAACGTTAGGGGGGGGGGAGGG
AGAGGGGCGGAATT (SEQ ID NO: 6).

40. The method of producing a heterologous polypeptide in a transgenic plant of claim 30, wherein the 3' UTR of a first positive strand single-stranded RNA virus is a 3' UTR of a first positive strand single-stranded RNA virus having no DNA stage.

41. The method of producing a heterologous polypeptide in a transgenic plant of claim 40, wherein the 3' UTR of a first positive strand single-stranded RNA virus having no DNA stage is a 3' UTR of a first bromovirus.

42. The method of producing a heterologous polypeptide in a transgenic plant of claim 41, wherein the 3' UTR of a first bromovirus is a 3' UTR of a first Cowpea chlorotic mottle virus.

43. The method of producing a heterologous polypeptide in a transgenic plant of claim 42, wherein a DNA copy of the 3' UTR of a first Cowpea chlorotic mottle virus comprises

the sequence:

AGTGCCCGCTGAAGAGCGTTACACTAGTGTGGCCTACTTGAAGGCTAGTTATAACCG

TTTCTTTAAACGGTAATCGTTGTTGAAACGTCTTCCTTTTACAAGAGGATTGAGCTGC

CCTTGGGTTTTACTCCTTGAACCCTTCGGAAGAACTCTTTGGAGTTCGTACCAGTACC

TCACATAGTGAGGTAATAAGACTGGTGGGCAGCGCCTAGTCGAAAGACTAGGTGAT

CTCTAAGGAGACC (SEQ ID NO: 8).

44. The method of producing a heterologous polypeptide in a transgenic plant of claim 30, further comprising a sequence complementary to an intron.

45. The method of producing a heterologous polypeptide in a transgenic plant of claim 30, further comprising a transcription termination signal.

46. The method of producing a heterologous polypeptide in a transgenic plant of claim 30, wherein the plant is a dicotyledonous plant.

47. The method of producing a heterologous polypeptide in a transgenic plant of claim 46, wherein the dicotyledonous plant is a *Nicotiana* plant.

48. The method of producing a heterologous polypeptide in a transgenic plant of claim 47, wherein the *Nicotiana* plant is a *Nicotiana benthamiana* plant.

49. The method of producing a heterologous polypeptide in a transgenic plant of claim 30, wherein the providing a stimulus to the transgenic plant for synthesis of an RNA complementary to an RNA transcript of the recombinant DNA comprises infecting the transgenic plant with a second positive strand single-stranded RNA virus.

50. The method of producing a heterologous polypeptide in a transgenic plant of claim 49, wherein the second positive strand single-stranded RNA virus is a positive strand single-stranded RNA virus having no DNA stage.

51. The method of producing a heterologous polypeptide in a transgenic plant of claim 50, wherein the second positive strand single-stranded RNA virus having no DNA stage is selected from the group consisting of a positive strand single-stranded RNA plant virus having no DNA stage and a positive single-stranded RNA animal virus having no DNA stage.

52. The method of producing a heterologous polypeptide in a transgenic plant of claim 51, wherein the second positive strand single-stranded RNA plant virus having no DNA stage is selected from the group consisting of a second Bromovirus, a Tobacco etch virus, a Tobacco vein mottle virus, and a Pepper mottle virus.

53. The method of producing a heterologous polypeptide in a transgenic plant of claim 52, wherein the second Bromovirus is selected from a second Cowpea chlorotic mottle virus and a second Brome mosaic virus.

54. The method of producing a heterologous polypeptide in a transgenic plant of claim 53, wherein the second Bromovirus is a second Cowpea chlorotic mottle virus.

55. The method of producing a heterologous polypeptide in a transgenic plant of claim 30, wherein the providing a stimulus to the cell for synthesis of an RNA complementary to an RNA transcript of the recombinant DNA comprises transfecting the transgenic plant with a cDNA of a second positive strand single-stranded RNA virus.

56. The method of producing a heterologous polypeptide in a transgenic plant of claim 55, wherein the cDNA of a second positive strand single-stranded RNA virus comprises a cDNA encoding an RNA dependent RNA polymerase.

57. The method of producing a heterologous polypeptide in a transgenic plant of claim 56, wherein the second positive strand single-stranded RNA virus is a positive strand single-stranded RNA virus having no DNA stage.

58. The method of producing a heterologous polypeptide in a transgenic plant of claim 57, wherein the second positive strand single-stranded RNA virus having no DNA stage is selected from the group consisting of a positive strand single-stranded RNA plant virus having no DNA stage and a positive single-stranded RNA animal virus having no DNA stage.

59. The method of producing a heterologous polypeptide in a transgenic plant of claim 58, wherein the second positive strand single-stranded RNA plant virus having no DNA stage is selected from the group consisting of a second Bromovirus, a Tobacco etch virus, a Tobacco vein mottle virus, and a Pepper mottle virus.

60. The method of producing a heterologous polypeptide in a transgenic plant of claim 59, wherein the second positive strand single-stranded RNA plant virus having no DNA stage is selected from the group consisting of a second Cowpea chlorotic mottle virus, a second Brome mosaic virus, a second Tobacco etch virus, a second Tobacco vein mottle virus, and a second Pepper mottle virus.

61. The method of producing a heterologous polypeptide in a transgenic plant of claim 60, wherein the second Bromovirus is selected from a second Cowpea chlorotic mottle virus and a Brome mosaic virus.

62. The method of producing a heterologous polypeptide in a transgenic plant of claim 30, wherein the providing a stimulus to the cell for synthesis of an RNA complementary to an RNA transcript of the recombinant DNA comprises transfecting the transgenic plant with RNA of a second positive strand single-stranded RNA virus, the RNA comprising at least one sequence encoding a polypeptide component of an RNA virus replication complex.

63. The method of producing a heterologous polypeptide in a transgenic plant of claim 62, wherein the RNA comprising at least one sequence encoding a polypeptide component

of an RNA virus replication complex is an RNA comprising a sequence encoding an RNA-dependent RNA polymerase.

64. The method of producing a heterologous polypeptide in a transgenic plant of claim 63, wherein the second positive strand single-stranded RNA virus is a positive strand single-stranded RNA virus having no DNA stage.

65. The method of producing a heterologous polypeptide in a transgenic plant of claim 64, wherein the second positive strand single-stranded RNA virus having no DNA stage is selected from the group consisting of a positive strand single-stranded RNA plant virus having no DNA stage and a positive single-stranded RNA animal virus having no DNA stage.

66. The method of producing a heterologous polypeptide in a transgenic plant of claim 65, wherein the second positive strand single-stranded RNA plant virus having no DNA stage is selected from the group consisting of a second Bromovirus, a Tobacco etch virus, a Tobacco vein mottle virus, and a Pepper mottle virus.

67. The method of producing a heterologous polypeptide in a transgenic plant of claim 66, wherein the second Bromovirus is selected from a second Cowpea chlorotic mottle virus and a second Brome mosaic virus.

68. The method of producing a heterologous polypeptide in a transgenic plant of claim 67, wherein the second Bromovirus is a second Cowpea chlorotic mottle virus.

69. The method of producing a heterologous polypeptide in a transgenic plant of claim 30, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 50:1.

70. The method of producing a heterologous polypeptide in a transgenic plant of claim 69, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 100:1.

71. The method of producing a heterologous polypeptide in a transgenic plant of claim 70, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 1000:1.

72. The method of producing a heterologous polypeptide in a transgenic plant of claim 71, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 10,000:1.

73. A method of producing a heterologous polypeptide in a transgenic cell, the method comprising:

a) providing a cell comprising a recombinant DNA molecule comprising a promoter operably linked to a DNA sequence comprising, in the 5' to 3' direction,

i) a sequence complementary to a coding sequence for a heterologous polypeptide;

ii) a sequence complementary to an internal ribosome entry site;

iii) a 3' UTR of a first positive strand single-stranded RNA virus; and

b) providing a stimulus to the cell for synthesis of an RNA complementary to an RNA transcript of the recombinant DNA.

74. The method of producing a heterologous polypeptide in a transgenic cell of claim 73, wherein the promoter is a selected from the group consisting of a constitutive promoter and an inducible promoter.

75. The method of producing a heterologous polypeptide in a transgenic cell of claim 74, wherein the promoter is a constitutive promoter.

76. The method of producing a heterologous polypeptide in a transgenic cell of claim 75, wherein the constitutive promoter is a eukaryotic constitutive promoter selected from the group consisting of a cauliflower mosaic virus 35S promoter, a blueberry red ringspot virus promoter, a ubiquitin gene promoter, an actin gene promoter, an NeIF-4A10 promoter, a maize Adh1-based pEmu promoter, a barley leaf thionin BTH6 promoter, a cassava vein mosaic virus promoter, a sugarcane bacilliform badnavirus promoter and a histone gene promoter.

77. The method of producing a heterologous polypeptide in a transgenic cell of claim 76, wherein the eukaryotic constitutive promoter is a cauliflower mosaic virus 35S promoter.

78. The method of producing a heterologous polypeptide in a transgenic plant of claim 77, wherein the cauliflower mosaic virus 35S promoter comprises the sequence:

AGATTAGCCTTTTCAATTTCAGAAAGAATGCTAACCCACAGATGGTTAGAGAGGCTT
ACGCAGCAGGTCTCATCAAGACGATCTACCCGAGCAATAATCTCCAGGAAATCAAA
TACCTTCCCAAGAAGGTTAAAGATGCAGTCAAAAGATTCAGGACTAACTGCATCAA
GAACACAGAGAAAGATATATTTCTCAAGATCAGAAGTACTATTCCAGTATGGACGA
TTCAAGGCTTGCTTCACAAACCAAGGCAAGTAATAGAGATTGGAGTCTCTAAAAAG
GTAGTTCCCACTGAATCAAAGGCCATGGAGTCAAAGATTCAAATAGAGGACCTAAC
AGAACTCGCCGTAAAGACTGGCGAACAGTTCATACAGAGTCTCTTACGACTCAATG
ACAAGAAGAAAATCTTCGTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAA
AATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAG
GGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTG
AAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAA

GGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCA
CGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGAT
TGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAA
GACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGAACACG (SEQ ID NO: 3).

79. The method of producing a heterologous polypeptide in a transgenic cell of claim 73, wherein the coding sequence for a heterologous polypeptide encodes a polypeptide selected from the group consisting of a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

80. The method of producing a heterologous polypeptide in a transgenic cell of claim 73, wherein the sequence complementary to an IRES is a sequence complementary to an IRES selected from the group consisting of a picornavirus IRES, a foot-and-mouth disease virus IRES, an encephalomyocarditis virus IRES, a hepatitis A virus IRES, a hepatitis C virus IRES, a human rhinovirus IRES, a poliovirus IRES, a swine vesicular disease virus IRES, a turnip mosaic potyvirus IRES, a human fibroblast growth factor 2 mRNA IRES, a pestivirus IRES, a Leishmania RNA virus IRES, a Moloney murine leukemia virus IRES a human rhinovirus 14 IRES, an aphthovirus IRES, a human immunoglobulin heavy chain binding protein mRNA IRES, a *Drosophila* Antennapedia mRNA IRES, a human fibroblast growth factor 2 mRNA IRES, a hepatitis G virus IRES, a tobamovirus IRES, a vascular endothelial growth factor mRNA IRES, a Coxsackie B group virus IRES, a c-myc protooncogene mRNA IRES, a human MYT2 mRNA IRES, a human parechovirus type 1 virus IRES, a human parechovirus type 2 virus IRES, a eukaryotic initiation factor 4GI mRNA IRES, a Plautia stali intestine virus IRES, a Theiler's murine encephalomyelitis virus IRES, a bovine enterovirus IRES, a connexin 43 mRNA IRES, a homeodomain protein Gtx mRNA IRES, an AML1 transcription factor mRNA

IRES, an NF-kappa B repressing factor mRNA IRES, an X-linked inhibitor of apoptosis mRNA IRES, a cricket paralysis virus RNA IRES, a p58(PITSLRE) protein kinase mRNA IRES, an ornithine decarboxylase mRNA IRES, a connexin-32 mRNA IRES, a bovine viral diarrhea virus IRES, an insulin-like growth factor I receptor mRNA IRES, a human immunodeficiency virus type 1 gag gene IRES, a classical swine fever virus IRES, a Kaposi's sarcoma-associated herpes virus IRES, a short IRES selected from a library of random oligonucleotides, a Jembrana disease virus IRES, an apoptotic protease-activating factor 1 mRNA IRES, a Rhopalosiphum padi virus IRES, a cationic amino acid transporter mRNA IRES, a human insulin-like growth factor II leader 2 mRNA IRES, a giardiavirus IRES, a Smad5 mRNA IRES, a porcine teschovirus-1 talfan IRES, a *Drosophila* Hairless mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2 mRNA IRES, an Epstein-Barr virus IRES, a hibiscus chlorotic ringspot virus IRES, a rat pituitary vasopressin V1b receptor mRNA IRES, and a human hsp70 mRNA IRES.

81. The method of producing a heterologous polypeptide in a transgenic cell of claim 80, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to a picornavirus internal ribosome entry site.

82. The method of producing a heterologous polypeptide in a transgenic cell of claim 81, wherein the sequence complementary to a picornavirus internal ribosome entry site comprises the sequence:

TTATCATCGTGTTTTTCAAAGGAAAACCACGTCCCCGTGGTTCGGGGGGCCTAGACG
TTTTTTTAACCTCGACTAAACACATGTAAAGCATGTGCACCGAGGCCCCAGATCAGA
TCCCATACAATGGGGTACCTTCTGGGCATCCTTCAGCCCCTTGTTGAATACGCTTGA
GGAGAGCCATTTGACTCTTTCCACAACTATCCAACTCACAACGTGGCACTGGGGTTG
TGCCGCCTTTGCAGGTGTATCTTATACACGTGGCTTTTGGCCGCAGAGGCACCTGTC

GCCAGGTGGGGGGTTCCGCTGCCTGCAAAGGGTCGCTACAGACGTTGTTTGTCTTCA
AGAAGCTTCCAGAGGAACTGCTTCCTTCACGACATTCAACAGACCTTGCATTCCTTT
GGCGAGAGGGGAAAGACCCCTAGGAATGCTCGTCAAGAAGACAGGGCCAGGTTTCC
GGGCCCTCACATTGCCAAAAGACGGCAATATGGTGGAAAATCACATATAGACAAAC
GCACACCGGCCTTATTCCAAGCGGCTTCGGCCAGTAACGTTAGGGGGGGGGGAGGG
AGAGGGGCGGAATT (SEQ ID NO: 6).

83. The method of producing a heterologous polypeptide in a transgenic cell of claim 73, wherein the 3' UTR of a first positive strand single-stranded RNA virus is a 3' UTR of a first positive strand single-stranded RNA virus having no DNA stage.

84. The method of producing a heterologous polypeptide in a transgenic cell of claim 83, wherein the 3' UTR of a first positive strand single-stranded RNA virus having no DNA stage is a 3' UTR of a first bromovirus.

85. The method of producing a heterologous polypeptide in a transgenic cell of claim 84, wherein the 3' UTR of a first bromovirus is a 3' UTR of a first Cowpea chlorotic mottle virus.

86. The method of producing a heterologous polypeptide in a transgenic cell of claim 85, wherein a DNA copy of the 3' UTR of a first Cowpea chlorotic mottle virus comprises the sequence:

AGTGCCCGCTGAAGAGCGTTACACTAGTGTGGCCTACTTGAAGGCTAGTTATAACCG
TTTCTTTAAACGGTAATCGTTGTTGAAACGTCTTCCTTTTACAAGAGGATTGAGCTGC
CCTTGGGTTTTACTCCTTGAACCCTTCGGAAGAACTCTTTGGAGTTCGTACCAGTACC
TCACATAGTGAGGTAATAAGACTGGTGGGCAGCGCCTAGTCGAAAGACTAGGTGAT
CTCTAAGGAGACC (SEQ ID NO: 8).

87. The method of producing a heterologous polypeptide in a transgenic cell of claim 73, further comprising a sequence complementary to an intron.

88. The method of producing a heterologous polypeptide in a transgenic cell of claim 73, further comprising a transcription termination signal.

89. The method of producing a heterologous polypeptide in a transgenic cell of claim 73, wherein the recombinant DNA molecule is comprised by a host cell.

90. The method of producing a heterologous polypeptide in a transgenic cell of claim 89, wherein the host cell is a plant cell.

91. The method of producing a heterologous polypeptide in a transgenic cell of claim 90, wherein the plant cell is comprised by a plant.

92. The method of producing a heterologous polypeptide in a transgenic cell of claim 91, wherein the plant is a dicotyledonous plant.

93. The method of producing a heterologous polypeptide in a transgenic cell of claim 92, wherein the dicotyledonous plant is a *Nicotiana* plant.

94. The method of producing a heterologous polypeptide in a transgenic cell of claim 93, wherein the *Nicotiana* plant is a *Nicotiana benthamiana* plant.

95. The method of producing a heterologous polypeptide in a transgenic cell of claim 73, wherein the providing a stimulus to the cell for synthesis of an RNA complementary to an RNA transcript of the recombinant DNA comprises infecting the transgenic cell with a second positive strand single-stranded RNA virus.

96. The method of producing a heterologous polypeptide in a transgenic cell of claim 95, wherein the second positive strand single-stranded RNA virus is a positive strand single-stranded RNA virus having no DNA stage.

97. The method of producing a heterologous polypeptide in a transgenic cell of claim 96, wherein the second positive strand single-stranded RNA virus having no DNA stage is selected from the group consisting of a positive strand single-stranded RNA plant virus having no DNA stage and a positive single-stranded RNA animal virus having no DNA stage.

98. The method of producing a heterologous polypeptide in a transgenic cell of claim 97, wherein the second positive strand single-stranded RNA plant virus having no DNA stage is selected from the group consisting of a second Bromovirus, a Tobacco etch virus, a Tobacco vein mottle virus, and a Pepper mottle virus.

99. The method of producing a heterologous polypeptide in a transgenic cell of claim 98, wherein the second Bromovirus is selected from a second Cowpea chlorotic mottle virus and a second Brome mosaic virus.

100. The method of producing a heterologous polypeptide in a transgenic cell of claim 99, wherein the second Bromovirus is a second Cowpea chlorotic mottle virus.

101. The method of producing a heterologous polypeptide in a transgenic cell of claim 73, wherein the providing a stimulus to the cell for synthesis of an RNA complementary to an RNA transcript of the recombinant DNA comprises transfecting the transgenic cell with a cDNA of a second positive strand single-stranded RNA virus.

102. The method of producing a heterologous polypeptide in a transgenic cell of claim 101, wherein the cDNA of a second positive strand single-stranded RNA virus comprises a cDNA encoding an RNA dependent RNA polymerase.

103. The method of producing a heterologous polypeptide in a transgenic cell of claim 101, wherein the second positive strand single-stranded RNA virus is a positive strand single-stranded RNA virus having no DNA stage.

104. The method of producing a heterologous polypeptide in a transgenic cell of claim 103, wherein the second positive strand single-stranded RNA virus having no DNA stage is selected from the group consisting of a positive strand single-stranded RNA plant virus having no DNA stage and a positive single-stranded RNA animal virus having no DNA stage.

105. The method of producing a heterologous polypeptide in a transgenic cell of claim 104, wherein the second positive strand single-stranded RNA plant virus having no DNA stage is selected from the group consisting of a second Bromovirus, a Tobacco etch virus, a Tobacco vein mottle virus, and a Pepper mottle virus.

106. The method of producing a heterologous polypeptide in a transgenic cell of claim 105, wherein the second positive strand single-stranded RNA plant virus having no DNA stage is selected from the group consisting of a second Cowpea chlorotic mottle virus, a second Brome mosaic virus, a second Tobacco etch virus, a second Tobacco vein mottle virus, and a second Pepper mottle virus.

107. The method of producing a heterologous polypeptide in a transgenic cell of claim 106, wherein the second Bromovirus is selected from a second Cowpea chlorotic mottle virus and a Brome mosaic virus.

108. The method of producing a heterologous polypeptide in a transgenic cell of claim 73, wherein the providing a stimulus to the cell for synthesis of an RNA complementary to an RNA transcript of the recombinant DNA comprises transfecting the transgenic cell with RNA of a second positive strand single-stranded RNA virus, the RNA comprising at least one sequence encoding a polypeptide component of an RNA virus replication complex.

109. The method of producing a heterologous polypeptide in a transgenic cell of claim 108, wherein the RNA comprising at least one sequence encoding a polypeptide component of

an RNA virus replication complex is an RNA comprising a sequence encoding an RNA-dependent RNA polymerase.

110. The method of producing a heterologous polypeptide in a transgenic cell of claim 109, wherein the second positive strand single-stranded RNA virus is a positive strand single-stranded RNA virus having no DNA stage.

111. The method of producing a heterologous polypeptide in a transgenic cell of claim 110, wherein the second positive strand single-stranded RNA virus having no DNA stage is selected from the group consisting of a positive strand single-stranded RNA plant virus having no DNA stage and a positive single-stranded RNA animal virus having no DNA stage.

112. The method of producing a heterologous polypeptide in a transgenic cell of claim 111, wherein the second positive strand single-stranded RNA plant virus having no DNA stage is selected from the group consisting of a second Bromovirus, a Tobacco etch virus, a Tobacco vein mottle virus, and a Pepper mottle virus.

113. The method of producing a heterologous polypeptide in a transgenic cell of claim 112, wherein the second Bromovirus is selected from a second Cowpea chlorotic mottle virus and a second Brome mosaic virus.

114. The method of producing a heterologous polypeptide in a transgenic cell of claim 113, wherein the second Bromovirus is a second Cowpea chlorotic mottle virus.

115. The method of producing a heterologous polypeptide in a transgenic cell of claim 73, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 50:1.

116. The method of producing a heterologous polypeptide in a transgenic cell of claim 115, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 100:1.

117. The method of producing a heterologous polypeptide in a transgenic cell of claim 116, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 1000:1.

118. The method of producing a heterologous polypeptide in a transgenic cell of claim 117, wherein the molar concentration ratio of heterologous polypeptide in a cell provided a stimulus relative to a cell not provided a stimulus is at least about 10,000:1.

119. A recombinant DNA molecule comprising a promoter operably linked to a DNA sequence comprising, in the 5' to 3' direction:

a) a sequence complementary to a coding sequence for a heterologous polypeptide;

b) a sequence complementary to an internal ribosome entry site; and

c) a 3' UTR of a positive strand single-stranded RNA virus.

120. The recombinant DNA molecule of claim 119, wherein the promoter is a selected from the group consisting of a constitutive promoter and an inducible promoter.

121. The recombinant DNA molecule of claim 120, wherein the promoter is a constitutive promoter.

122. The recombinant DNA molecule of claim 121, wherein the constitutive promoter is a eukaryotic constitutive promoter selected from the group consisting of a cauliflower mosaic virus 35S promoter, a blueberry red ringspot virus promoter, a ubiquitin gene promoter, an actin gene promoter, an NeIF-4A10 promoter, a maize Adh1-based pEmu promoter, a barley leaf

thionin BTH6 promoter, a cassava vein mosaic virus promoter, a sugarcane bacilliform badnavirus promoter and a histone gene promoter.

123. The recombinant DNA molecule of claim 122, wherein the eukaryotic constitutive promoter is a cauliflower mosaic virus 35S promoter.

124. The recombinant DNA molecule of claim 123, wherein the cauliflower mosaic virus 35S promoter comprises the sequence:

AGATTAGCCTTTTCAATTTCAGAAAGAATGCTAACCCACAGATGGTTAGAGAGGCTT
ACGCAGCAGGTCTCATCAAGACGATCTACCCGAGCAATAATCTCCAGGAAATCAAA
TACCTTCCCAAGAAGGTTAAAGATGCAGTCAAAAGATTCAGGACTAACTGCATCAA
GAACACAGAGAAAGATATATTTCTCAAGATCAGAAGTACTATTCCAGTATGGACGA
TTCAAGGCTTGCTTCACAAACCAAGGCAAGTAATAGAGATTGGAGTCTCTAAAAAG
GTAGTTCCCACTGAATCAAAGGCCATGGAGTCAAAGATTCAAATAGAGGACCTAAC
AGAACTCGCCGTAAAGACTGGCGAACAGTTCATACAGAGTCTCTTACGACTCAATG
ACAAGAAGAAAATCTTCGTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAA
AATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAG
GGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTG
AAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAA
GGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCA
CGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGAT
TGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAA
GACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGAACACG (SEQ ID NO: 3).

125. The recombinant DNA molecule of claim 119, wherein the coding sequence for a heterologous polypeptide encodes a polypeptide selected from the group consisting of a

hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

126. The recombinant DNA molecule of claim 119, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to an IRES selected from the group consisting of a picornavirus IRES, a foot-and-mouth disease virus IRES, an encephalomyocarditis virus IRES, a hepatitis A virus IRES, a hepatitis C virus IRES, a human rhinovirus IRES, a poliovirus IRES, a swine vesicular disease virus IRES, a turnip mosaic potyvirus IRES, a human fibroblast growth factor 2 mRNA IRES, a pestivirus IRES, a Leishmania RNA virus IRES, a Moloney murine leukemia virus IRES a human rhinovirus 14 IRES, an aphthovirus IRES, a human immunoglobulin heavy chain binding protein mRNA IRES, a *Drosophila* Antennapedia mRNA IRES, a human fibroblast growth factor 2 mRNA IRES, a hepatitis G virus IRES, a tobamovirus IRES, a vascular endothelial growth factor mRNA IRES, a Coxsackie B group virus IRES, a c-myc protooncogene mRNA IRES, a human MYT2 mRNA IRES, a human parechovirus type 1 virus IRES, a human parechovirus type 2 virus IRES, a eukaryotic initiation factor 4GI mRNA IRES, a Plautia stali intestine virus IRES, a Theiler's murine encephalomyelitis virus IRES, a bovine enterovirus IRES, a connexin 43 mRNA IRES, a homeodomain protein Gtx mRNA IRES, an AML1 transcription factor mRNA IRES, an NF-kappa B repressing factor mRNA IRES, an X-linked inhibitor of apoptosis mRNA IRES, a cricket paralysis virus RNA IRES, a p58(PITSLRE) protein kinase mRNA IRES, an ornithine decarboxylase mRNA IRES, a connexin-32 mRNA IRES, a bovine viral diarrhea virus IRES, an insulin-like growth factor I receptor mRNA IRES, a human immunodeficiency virus type 1 gag gene IRES, a classical swine fever virus IRES, a Kaposi's sarcoma-associated herpes virus IRES, a short IRES selected from a library of random oligonucleotides, a Jembrana disease

virus IRES, an apoptotic protease-activating factor 1 mRNA IRES, a Rhopalosiphum padi virus IRES, a cationic amino acid transporter mRNA IRES, a human insulin-like growth factor II leader 2 mRNA IRES, a giardiavirus IRES, a Smad5 mRNA IRES, a porcine teschovirus-1 talfan IRES, a *Drosophila* Hairless mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2 mRNA IRES, an Epstein-Barr virus IRES, a hibiscus chlorotic ringspot virus IRES, a rat pituitary vasopressin V1b receptor mRNA IRES, and a human hsp70 mRNA IRES.

127. The recombinant DNA molecule of claim 126, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to a picornavirus internal ribosome entry site.

128. The recombinant DNA molecule of claim 127, wherein the sequence complementary to a picornavirus internal ribosome entry site comprises the sequence:
TTATCATCGTGTTTTTCAAAGGAAAACCACGTCCCCGTGGTTCGGGGGGCCTAGACG
TTTTTTTAACCTCGACTAAACACATGTAAAGCATGTGCACCGAGGCCCCAGATCAGA
TCCCATACAATGGGGTACCTTCTGGGCATCCTTCAGCCCCTTGTTGAATACGCTTGA
GGAGAGCCATTTGACTCTTTCCACAACTATCCAACTCACAACGTGGCACTGGGGTTG
TGCCGCCTTTGCAGGTGTATCTTATACACGTGGCTTTTGGCCGCAGAGGCACCTGTC
GCCAGGTGGGGGGTTCCGCTGCCTGCAAAGGGTCGCTACAGACGTTGTTTGTCTTCA
AGAAGCTTCCAGAGGAACTGCTTCCTTCACGACATTCAACAGACCTTGCATTCCTTT
GGCGAGAGGGGAAAGACCCCTAGGAATGCTCGTCAAGAAGACAGGGCCAGGTTTCC
GGGCCCTCACATTGCCAAAAGACGGCAATATGGTGGAAAATCACATATAGACAAAC
GCACACCGGCCTTATTCCAAGCGGCTTCGGCCAGTAACGTTAGGGGGGGGGGAGGG
AGAGGGGCGGAATT (SEQ ID NO: 6).

129. The recombinant DNA molecule of claim 119, wherein the 3' UTR of a positive strand single-stranded RNA virus is a 3' UTR of a positive strand single-stranded RNA virus having no DNA stage.

130. The recombinant DNA molecule of claim 129, wherein the 3' UTR of a positive strand single-stranded RNA virus having no DNA stage is a 3' UTR of a bromovirus.

131. The recombinant DNA molecule of claim 130, wherein the 3' UTR of a bromovirus is a 3' UTR of a Cowpea chlorotic mottle virus.

132. The recombinant DNA molecule of claim 131, wherein a DNA copy of the 3' UTR of a Cowpea chlorotic mottle virus comprises the sequence:

AGTGCCCGCTGAAGAGCGTTACACTAGTGTGGCCTACTTGAAGGCTAGTTATAACCG
TTTCTTTAAACGGTAATCGTTGTTGAAACGTCTTCCTTTTACAAGAGGATTGAGCTGC
CCTTGGGTTTTACTCCTTGAACCCTTCGGAAGAACTCTTTGGAGTTCGTACCAGTACC
TCACATAGTGAGGTAATAAGACTGGTGGGCAGCGCCTAGTCGAAAGACTAGGTGAT
CTCTAAGGAGACC (SEQ ID NO: 8).

133. The recombinant DNA molecule of claim 119, further comprising a sequence complementary to an intron.

134. The recombinant DNA molecule of claim 119, further comprising a transcription termination signal.

135. A transgenic host cell comprising the recombinant DNA molecule of claim 119.

136. The transgenic host cell of claim 134, wherein the transgenic host cell is a transgenic plant cell.

137. A transgenic plant comprising the transgenic plant cell of claim 136.

138. The transgenic plant of claim 137, wherein the transgenic plant is a transgenic dicotyledonous plant.

139. The transgenic dicotyledonous plant of claim 138, wherein the transgenic dicotyledonous plant is a transgenic *Nicotiana* plant.

140. The transgenic *Nicotiana* plant of claim 139, wherein the transgenic *Nicotiana* plant is a transgenic *Nicotiana benthamiana* plant.

141. Transgenic seed comprising the recombinant DNA molecule of claim 119.

142. A recombinant RNA molecule comprising, in the 5' to 3' direction:

a) an RNA sequence comprising a sequence complementary to a coding sequence for a heterologous polypeptide;

b) a sequence complementary to an internal ribosome entry site; and

c) a 3' UTR of a positive strand single-stranded RNA virus.

143. The recombinant RNA molecule of claim 142, wherein the coding sequence for a heterologous polypeptide encodes a polypeptide selected from the group consisting of a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

144. The recombinant RNA molecule of claim 142, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to an IRES selected from the group consisting of a picornavirus IRES, a foot-and-mouth disease virus IRES, an encephalomyocarditis virus IRES, a hepatitis A virus IRES, a hepatitis C virus IRES, a human rhinovirus IRES, a poliovirus IRES, a swine vesicular disease virus IRES, a turnip mosaic potyvirus IRES, a human fibroblast growth factor 2 mRNA IRES, a pestivirus IRES, a Leishmania RNA virus IRES, a Moloney murine leukemia virus IRES a human rhinovirus 14

IRES, an aphthovirus IRES, a human immunoglobulin heavy chain binding protein mRNA IRES, a *Drosophila* Antennapedia mRNA IRES, a human fibroblast growth factor 2 mRNA IRES, a hepatitis G virus IRES, a tobamovirus IRES, a vascular endothelial growth factor mRNA IRES, a Coxsackie B group virus IRES, a c-myc protooncogene mRNA IRES, a human MYT2 mRNA IRES, a human parechovirus type 1 virus IRES, a human parechovirus type 2 virus IRES, a eukaryotic initiation factor 4GI mRNA IRES, a Plautia stali intestine virus IRES, a Theiler's murine encephalomyelitis virus IRES, a bovine enterovirus IRES, a connexin 43 mRNA IRES, a homeodomain protein Gtx mRNA IRES, an AML1 transcription factor mRNA IRES, an NF-kappa B repressing factor mRNA IRES, an X-linked inhibitor of apoptosis mRNA IRES, a cricket paralysis virus RNA IRES, a p58(PITSLRE) protein kinase mRNA IRES, an ornithine decarboxylase mRNA IRES, a connexin-32 mRNA IRES, a bovine viral diarrhea virus IRES, an insulin-like growth factor I receptor mRNA IRES, a human immunodeficiency virus type 1 gag gene IRES, a classical swine fever virus IRES, a Kaposi's sarcoma-associated herpes virus IRES, a short IRES selected from a library of random oligonucleotides, a Jembrana disease virus IRES, an apoptotic protease-activating factor 1 mRNA IRES, a Rhopalosiphum padi virus IRES, a cationic amino acid transporter mRNA IRES, a human insulin-like growth factor II leader 2 mRNA IRES, a giardiavirus IRES, a Smad5 mRNA IRES, a porcine teschovirus-1 talfan IRES, a *Drosophila* Hairless mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2 mRNA IRES, an Epstein-Barr virus IRES, a hibiscus chlorotic ringspot virus IRES, a rat pituitary vasopressin V1b receptor mRNA IRES, and a human hsp70 mRNA IRES.

145. The recombinant RNA molecule of claim 144, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to a picornavirus internal ribosome entry site.

146. The recombinant RNA molecule of claim 145, wherein the sequence complementary to a picornavirus internal ribosome entry site comprises the sequence:

UUAUCAUCGUGUUUUUCAAAGGAAAACCACGUCCCCGUGGUUCGGGGGGCCUAG

ACGUUUUUUUUAACCUCGACUAAACACAUGUAAAGCAUGUGCACCGAGGCCCCAGA

UCAGAUCCCAUACAAUGGGGUACCUUCUGGGCAUCCUUCAGCCCCUUGUUGAAUA

CGCUUGAGGAGAGCCAUUUGACUCUUUCCACAACUAUCCAACUCACAACGUGGCA

CUGGGGUUGUGCCGCCUUUGCAGGUGUAUCUUAUACACGUGGCUUUUGGCCGCA

GAGGCACCUGUCGCCAGGUGGGGGGUUCCGCUGCCUGCAAAGGGUCGCUACAGAC

GUUGUUUGUCUUCAAGAAGCUUCCAGAGGAACUGCUUCCUUCACGACAUUCAACA

GACCUUGCAUUCCUUUGGCGAGAGGGGAAAGACCCCUAGGAAUGCUCGUCAAGA

AGACAGGGCCAGGUUUCCGGGCCCUCACAUUGCCAAAAGACGGCAAUAUGGUGGA

AAAUCACAUAUAGACAAACGCACACCGGCCUUAUUCCAAGCGGCUUCGGCCAGUA

ACGUUAGGGGGGGGGGAGGGAGAGGGGCGGAAUU (SEQ ID NO: 7).

147. The recombinant RNA molecule of claim 142, wherein the 3' UTR of a positive strand single-stranded RNA virus is a 3' UTR of a positive strand single-stranded RNA virus having no DNA stage.

148. The recombinant RNA molecule of claim 147, wherein the 3' UTR of a positive strand single-stranded RNA virus having no DNA stage is a 3' UTR of a bromovirus

149. The recombinant RNA molecule of claim 148, wherein the 3' UTR of a bromovirus is a 3' UTR of a Cowpea chlorotic mottle virus.

150. The recombinant RNA molecule of claim 149, wherein an RNA copy of the 3' UTR of a Cowpea chlorotic mottle virus comprises the sequence:

AGUGCCCGCUGAAGAGCGUUACACUAGUGUGGCCUACUUGAAGGCUAGUUAUAA

CCGUUUCUUUAAACGGUAAUCGUUGUUGAAACGUCUUCCUUUUACAAGAGGAUU

GAGCUGCCCUUGGGUUUUACUCCUUGAACCCUUCGGAAGAACUCUUUGGAGUUCG

UACCAGUACCUCACAUAGUGAGGUAAUAAGACUGGUGGGCAGCGCCUAGUCGAA

AGACUAGGUGAUCUCUAAGGAGACC (SEQ ID NO: 9).

151. The recombinant RNA molecule of claim 142, further comprising a sequence complementary to an intron.

152. A transgenic host cell comprising the recombinant RNA molecule of claim 142.

153. The transgenic host cell of claim 152, wherein the transgenic host cell is a transgenic plant cell.

154. A transgenic plant comprising the transgenic plant cell of claim 153.

155. The transgenic plant of claim 154, wherein the transgenic plant is a transgenic dicotyledonous plant.

156. The transgenic dicotyledonous plant 155, wherein the transgenic dicotyledonous plant is a transgenic *Nicotiana* plant.

157. The transgenic *Nicotiana* plant of claim 155, wherein the transgenic *Nicotiana* plant is a transgenic *Nicotiana benthamiana* plant.

158. Transgenic seed comprising the recombinant RNA of claim 142.

159. An RNA complement of a recombinant RNA molecule, the complement comprising, in the 5' to 3' direction:

a) a sequence complementary to a 3' UTR of a positive strand single-stranded RNA virus;

b) an internal ribosome entry site; and

c) an RNA sequence encoding a heterologous polypeptide.

160. The RNA complement of a recombinant RNA molecule of claim 159, wherein the RNA sequence encoding a heterologous polypeptide encodes a polypeptide selected from the group consisting of a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

161. The RNA complement of a recombinant RNA molecule of claim 159, wherein the internal ribosome entry site is selected from the group consisting of a picornavirus IRES, a foot-and-mouth disease virus IRES, an encephalomyocarditis virus IRES, a hepatitis A virus IRES, a hepatitis C virus IRES, a human rhinovirus IRES, a poliovirus IRES, a swine vesicular disease virus IRES, a turnip mosaic potyvirus IRES, a human fibroblast growth factor 2 mRNA IRES, a pestivirus IRES, a Leishmania RNA virus IRES, a Moloney murine leukemia virus IRES a human rhinovirus 14 IRES, an aphthovirus IRES, a human immunoglobulin heavy chain binding protein mRNA IRES, a *Drosophila* Antennapedia mRNA IRES, a human fibroblast growth factor 2 mRNA IRES, a hepatitis G virus IRES, a tobamovirus IRES, a vascular endothelial growth factor mRNA IRES, a Coxsackie B group virus IRES, a c-myc protooncogene mRNA IRES, a human MYT2 mRNA IRES, a human parechovirus type 1 virus IRES, a human parechovirus type 2 virus IRES, a eukaryotic initiation factor 4GI mRNA IRES, a Plautia stali intestine virus IRES, a Theiler's murine encephalomyelitis virus IRES, a bovine enterovirus IRES, a connexin 43 mRNA IRES, a homeodomain protein Gtx mRNA IRES, an AML1 transcription factor mRNA IRES, an NF-kappa B repressing factor mRNA IRES, an X-linked inhibitor of apoptosis mRNA IRES, a cricket paralysis virus RNA IRES, a p58(PITSLRE) protein kinase mRNA IRES, an ornithine decarboxylase mRNA IRES, a connexin-32 mRNA IRES, a bovine viral diarrhea virus IRES, an insulin-like growth factor I receptor mRNA IRES, a human immunodeficiency virus type 1 gag gene IRES, a classical swine fever virus IRES, a

Kaposi's sarcoma-associated herpes virus IRES, a short IRES selected from a library of random oligonucleotides, a Jembrana disease virus IRES, an apoptotic protease-activating factor 1 mRNA IRES, a Rhopalosiphum padi virus IRES, a cationic amino acid transporter mRNA IRES, a human insulin-like growth factor II leader 2 mRNA IRES, a giardiavirus IRES, a Smad5 mRNA IRES, a porcine teschovirus-1 talfan IRES, a *Drosophila* Hairless mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2 mRNA IRES, an Epstein-Barr virus IRES, a hibiscus chlorotic ringspot virus IRES, a rat pituitary vasopressin V1b receptor mRNA IRES, and a human hsp70 mRNA IRES.

162. The RNA complement of a recombinant RNA molecule of claim 161, wherein the internal ribosome entry site is a picornavirus internal ribosome entry site.

163. The RNA complement of a recombinant RNA molecule of claim 162, wherein the picornavirus internal ribosome entry site comprises the sequence:

AAUUCCGCCCCUCUCCCUCCCCCCCCCCUAACGUUACUGGCCGAAGCCGCUUGGAA
UAAGGCCGGUGUGCGUUUGUCUAUAUGUGAUUUUCCACCAUAUUGCCGUCUUUU
GGCAAUGUGAGGGCCCGGAAACCUGGCCCUGUCUUCUUGACGAGCAUUCCUAGGG
GUCUUUCCCCUCUCGCCAAAGGAAUGCAAGGUCUGUUGAAUGUCGUGAAGGAAG
CAGUUCCUCUGGAAGCUUCUUGAAGACAAACAACGUCUGUAGCGACCCUUUGCAG
GCAGCGGAACCCCCCACCUGGCGACAGGUGCCUCUGCGGCCAAAAGCCACGUGUA
UAAGAUACACCUGCAAAGGCGGCACAACCCCAGUGCCACGUUGUGAGUUGGAUAG
UUGUGGAAAGAGUCAAAUGGCUCUCCUCAAGCGUAUUCAACAAGGGGCUGAAGG
AUGCCCAGAAGGUACCCCAUUGUAUGGGAUCUGAUCUGGGGCCUCGGUGCACAUG
CUUUACAUGUGUUUAGUCGAGGUUAAAAAAACGUCUAGGCCCCCCGAACCACGGG
GACGUGGUUUUCCUUUGAAAAACACGAUGAUAA (SEQ ID NO: 5).

164. The RNA complement of a recombinant RNA molecule of claim 159, wherein the complement of a 3' UTR of a positive strand single-stranded RNA virus is a complement of a 3' UTR of a positive strand single-stranded RNA virus having no DNA stage.

165. The RNA complement of a recombinant RNA molecule of claim 164, wherein the complement of a 3' UTR of a positive strand single-stranded RNA virus having no DNA stage is a complement 3' UTR of a bromovirus

166. The RNA complement of a recombinant RNA molecule of claim 165, wherein the complement of a 3' UTR of a bromovirus is a complement of a 3' UTR of a Cowpea chlorotic mottle virus.

167. The RNA complement of a recombinant RNA molecule of claim 166, wherein the complement of a 3' UTR of a Cowpea chlorotic mottle virus comprises the sequence: GGUCUCCUUAGAGAUCACCUAGUCUUUCGACUAGGCGCUGCCCACCAGUCUUAUU ACCUCACUAUGUGAGGUACUGGUACGAACUCCAAAGAGUUCUUCCGAAGGGUUC AAGGAGUAAAACCCAAGGGCAGCUCAAUCCUCUUGUAAAAGGAAGACGUUUCAA CAACGAUUACCGUUUAAAGAAACGGUUAUAACUAGCCUUCAAGUAGGCCACACU AGUGUAACGCUCUUCAGCGGGCACU (SEQ ID NO: 11).

168. The RNA complement of a recombinant RNA molecule of claim 159, further comprising an intron.

169. A transgenic host cell comprising the RNA complement of a recombinant RNA molecule of claim 159.

170. The transgenic host cell of claim 169, wherein the transgenic host cell is a transgenic plant cell.

171. A transgenic plant comprising the transgenic plant cell of claim 170.

172. The transgenic plant of claim 171, wherein the transgenic plant is a transgenic dicotyledonous plant.

173. The transgenic dicotyledonous plant of claim 172, wherein the transgenic dicotyledonous plant is a transgenic *Nicotiana* plant.

174. The transgenic *Nicotiana* plant of claim 173, wherein the transgenic *Nicotiana* plant is a transgenic *Nicotiana benthamiana* plant.

175. Transgenic seed comprising the RNA complement of a recombinant RNA molecule of claim 159.

176. A recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell, the recombinant DNA molecule comprising a promoter operably linked, in the 5' to 3' direction, to DNA sequence comprising:

a) at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation;

b) a sequence complementary to an internal ribosome entry site; and

c) a 3' UTR of a positive strand single-stranded RNA virus.

177. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 176, wherein the promoter is a selected from the group consisting of a constitutive promoter and an inducible promoter.

178. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 177, wherein the promoter is a constitutive promoter.

179. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 178, wherein the constitutive promoter is a

eukaryotic constitutive promoter selected from the group consisting of a cauliflower mosaic virus 35S promoter, a blueberry red ringspot virus promoter, a ubiquitin gene promoter, an actin gene promoter, an NeIF-4A10 promoter, a maize Adh1-based pEmu promoter, a barley leaf thionin BTH6 promoter, a cassava vein mosaic virus promoter, a sugarcane bacilliform badnavirus promoter and a histone gene promoter.

180. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 179, wherein the eukaryotic constitutive promoter is a cauliflower mosaic virus 35S promoter.

181. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 180, wherein the cauliflower mosaic virus 35S promoter comprises the sequence:

AGATTAGCCTTTTCAATTTCAGAAAGAATGCTAACCCACAGATGGTTAGAGAGGCTT
ACGCAGCAGGTCTCATCAAGACGATCTACCCGAGCAATAATCTCCAGGAAATCAAA
TACCTTCCCAAGAAGGTTAAAGATGCAGTCAAAAGATTCAGGACTAACTGCATCAA
GAACACAGAGAAAGATATATTTCTCAAGATCAGAAGTACTATTCCAGTATGGACGA
TTCAAGGCTTGCTTCACAAACCAAGGCAAGTAATAGAGATTGGAGTCTCTAAAAAG
GTAGTTCCCACTGAATCAAAGGCCATGGAGTCAAAGATTCAAATAGAGGACCTAAC
AGAACTCGCCGTAAAGACTGGCGAACAGTTCATACAGAGTCTCTTACGACTCAATG
ACAAGAAGAAAATCTTCGTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAA
AATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAG
GGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTG
AAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAA
GGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCA

CGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGAT TGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAA GACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGAACACG (SEQ ID NO: 3).

182. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 176, wherein the coding sequence for a heterologous polypeptide encodes a polypeptide selected from the group consisting of a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

183. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 176, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to an IRES selected from the group consisting of a picornavirus IRES, a foot-and-mouth disease virus IRES, an encephalomyocarditis virus IRES, a hepatitis A virus IRES, a hepatitis C virus IRES, a human rhinovirus IRES, a poliovirus IRES, a swine vesicular disease virus IRES, a turnip mosaic potyvirus IRES, a human fibroblast growth factor 2 mRNA IRES, a pestivirus IRES, a Leishmania RNA virus IRES, a Moloney murine leukemia virus IRES a human rhinovirus 14 IRES, an aphthovirus IRES, a human immunoglobulin heavy chain binding protein mRNA IRES, a *Drosophila* Antennapedia mRNA IRES, a human fibroblast growth factor 2 mRNA IRES, a hepatitis G virus IRES, a tobamovirus IRES, a vascular endothelial growth factor mRNA IRES, a Coxsackie B group virus IRES, a c-myc protooncogene mRNA IRES, a human MYT2 mRNA IRES, a human parechovirus type 1 virus IRES, a human parechovirus type 2 virus IRES, a eukaryotic initiation factor 4GI mRNA IRES, a Plautia stali intestine virus IRES, a Theiler's murine encephalomyelitis virus IRES, a bovine enterovirus IRES, a connexin 43

mRNA IRES, a homeodomain protein Gtx mRNA IRES, an AML1 transcription factor mRNA IRES, an NF-kappa B repressing factor mRNA IRES, an X-linked inhibitor of apoptosis mRNA IRES, a cricket paralysis virus RNA IRES, a p58(PITSLRE) protein kinase mRNA IRES, an ornithine decarboxylase mRNA IRES, a connexin-32 mRNA IRES, a bovine viral diarrhea virus IRES, an insulin-like growth factor I receptor mRNA IRES, a human immunodeficiency virus type 1 gag gene IRES, a classical swine fever virus IRES, a Kaposi's sarcoma-associated herpes virus IRES, a short IRES selected from a library of random oligonucleotides, a Jembrana disease virus IRES, an apoptotic protease-activating factor 1 mRNA IRES, a Rhopalosiphum padi virus IRES, a cationic amino acid transporter mRNA IRES, a human insulin-like growth factor II leader 2 mRNA IRES, a giardiavirus IRES, a Smad5 mRNA IRES, a porcine teschovirus-1 talfan IRES, a *Drosophila* Hairless mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2 mRNA IRES, an Epstein-Barr virus IRES, a hibiscus chlorotic ringspot virus IRES, a rat pituitary vasopressin V1b receptor mRNA IRES, and a human hsp70 mRNA IRES.

184. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 183, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to a picornavirus internal ribosome entry site.

185. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 184, wherein the sequence complementary to a picornavirus internal ribosome entry site comprises the sequence:

TTATCATCGTGTTTTTCAAAGGAAAACCACGTCCCCGTGGTTCGGGGGGCCTAGACG
TTTTTTTAACCTCGACTAAACACATGTAAAGCATGTGCACCGAGGCCCCAGATCAGA
TCCCATACAATGGGGTACCTTCTGGGCATCCTTCAGCCCCTTGTTGAATACGCTTGA

GGAGAGCCATTTGACTCTTTCCACAACTATCCAACTCACAACGTGGCACTGGGGTTG
TGCCGCCTTTGCAGGTGTATCTTATACACGTGGCTTTTGGCCGCAGAGGCACCTGTC
GCCAGGTGGGGGGTTCCGCTGCCTGCAAAGGGTCGCTACAGACGTTGTTTGTCTTCA
AGAAGCTTCCAGAGGAACTGCTTCCTTCACGACATTCAACAGACCTTGCATTCCTTT
GGCGAGAGGGGAAAGACCCCTAGGAATGCTCGTCAAGAAGACAGGGCCAGGTTTCC
GGGCCCTCACATTGCCAAAAGACGGCAATATGGTGGAAAATCACATATAGACAAAC
GCACACCGGCCTTATTCCAAGCGGCTTCGGCCAGTAACGTTAGGGGGGGGGGAGGG
AGAGGGGCGGAATT (SEQ ID NO: 6).

186. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 176, wherein the 3' UTR of a positive strand single-stranded RNA virus is a 3' UTR of a positive strand single-stranded RNA virus having no DNA stage.

187. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 186, wherein the 3' UTR of a positive strand single-stranded RNA virus having no DNA stage is a 3' UTR of a bromovirus.

188. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 187, wherein the 3' UTR of a bromovirus is a 3' UTR of a Cowpea chlorotic mottle virus.

189. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 188, wherein a DNA copy of the 3' UTR of a Cowpea chlorotic mottle virus comprises the sequence:

AGTGCCCGCTGAAGAGCGTTACACTAGTGTGGCCTACTTGAAGGCTAGTTATAACCG
TTTCTTTAAACGGTAATCGTTGTTGAAACGTCTTCCTTTTACAAGAGGATTGAGCTGC

CCTTGGGTTTTACTCCTTGAACCCTTCGGAAGAACTCTTTGGAGTTCGTACCAGTACC TCACATAGTGAGGTAATAAGACTGGTGGGCAGCGCCTAGTCGAAAGACTAGGTGAT CTCTAAGGAGACC (SEQ ID NO: 8).

190. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 176, further comprising a sequence complementary to an intron.

191. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 176, further comprising a transcription termination signal.

192. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 176, wherein the at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation comprises a recombination site.

193. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 192, wherein the recombination site is selected from the group consisting of a bacteriophage lambda *att* site and a topoisomerase I-based recombination site.

194. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 176, wherein the at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation comprises at least one restriction enzyme recognition site.

195. The recombinant DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell of claim 176, wherein the at least one restriction enzyme recognition site comprises a polylinker.

196. A method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell, the method comprising:

a) providing a DNA molecule for construction of a vector for expressing a heterologous polypeptide in a transgenic cell, the DNA molecule comprising a promoter operably linked, in the 5' to 3' direction, to a DNA sequence comprising:

i) at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation;

ii) a sequence complementary to an internal ribosome entry site; and

iii) a 3' UTR of a positive strand single-stranded RNA virus; and

b) inserting a sequence encoding a heterologous polypeptide into the insertion site of the DNA molecule in an antisense orientation relative to the direction of transcription from the promoter.

197. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 196, wherein the promoter is a selected from the group consisting of a constitutive promoter and an inducible promoter.

198. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 197, wherein the promoter is a constitutive promoter.

199. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 198, wherein the constitutive promoter is a eukaryotic constitutive promoter selected from the group consisting of a cauliflower mosaic virus 35S

promoter, a blueberry red ringspot virus promoter, a ubiquitin gene promoter, an actin gene promoter, an NeIF-4A10 promoter, a maize Adh1-based pEmu promoter, a barley leaf thionin BTH6 promoter, a cassava vein mosaic virus promoter, a sugarcane bacilliform badnavirus promoter and a histone gene promoter.

200. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 199, wherein the eukaryotic constitutive promoter is a cauliflower mosaic virus 35S promoter.

201. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 200, wherein the cauliflower mosaic virus 35S promoter comprises the sequence:

AGATTAGCCTTTTCAATTTCAGAAAGAATGCTAACCCACAGATGGTTAGAGAGGCTT
ACGCAGCAGGTCTCATCAAGACGATCTACCCGAGCAATAATCTCCAGGAAATCAAA
TACCTTCCCAAGAAGGTTAAAGATGCAGTCAAAAGATTCAGGACTAACTGCATCAA
GAACACAGAGAAAGATATATTTCTCAAGATCAGAAGTACTATTCCAGTATGGACGA
TTCAAGGCTTGCTTCACAAACCAAGGCAAGTAATAGAGATTGGAGTCTCTAAAAAG
GTAGTTCCCACTGAATCAAAGGCCATGGAGTCAAAGATTCAAATAGAGGACCTAAC
AGAACTCGCCGTAAAGACTGGCGAACAGTTCATACAGAGTCTCTTACGACTCAATG
ACAAGAAGAAAATCTTCGTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAA
AATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAG
GGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTG
AAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAA
GGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCA
CGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGAT

TGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAA GACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGAACACG (SEQ ID NO: 3).

202. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 196, wherein the coding sequence for a heterologous polypeptide encodes a polypeptide selected from the group consisting of a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

203. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 196, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to an IRES selected from the group consisting of a picornavirus IRES, a foot-and-mouth disease virus IRES, an encephalomyocarditis virus IRES, a hepatitis A virus IRES, a hepatitis C virus IRES, a human rhinovirus IRES, a poliovirus IRES, a swine vesicular disease virus IRES, a turnip mosaic potyvirus IRES, a human fibroblast growth factor 2 mRNA IRES, a pestivirus IRES, a Leishmania RNA virus IRES, a Moloney murine leukemia virus IRES a human rhinovirus 14 IRES, an aphthovirus IRES, a human immunoglobulin heavy chain binding protein mRNA IRES, a *Drosophila* Antennapedia mRNA IRES, a human fibroblast growth factor 2 mRNA IRES, a hepatitis G virus IRES, a tobamovirus IRES, a vascular endothelial growth factor mRNA IRES, a Coxsackie B group virus IRES, a c-myc protooncogene mRNA IRES, a human MYT2 mRNA IRES, a human parechovirus type 1 virus IRES, a human parechovirus type 2 virus IRES, a eukaryotic initiation factor 4GI mRNA IRES, a Plautia stali intestine virus IRES, a Theiler's murine encephalomyelitis virus IRES, a bovine enterovirus IRES, a connexin 43 mRNA IRES, a homeodomain protein Gtx mRNA IRES, an AML1 transcription factor mRNA IRES, an NF-kappa B repressing factor mRNA IRES, an X-linked inhibitor of apoptosis mRNA IRES, a cricket paralysis virus RNA IRES, a

p58(PITSLRE) protein kinase mRNA IRES, an ornithine decarboxylase mRNA IRES, a connexin-32 mRNA IRES, a bovine viral diarrhea virus IRES, an insulin-like growth factor I receptor mRNA IRES, a human immunodeficiency virus type 1 gag gene IRES, a classical swine fever virus IRES, a Kaposi's sarcoma-associated herpes virus IRES, a short IRES selected from a library of random oligonucleotides, a Jembrana disease virus IRES, an apoptotic protease-activating factor 1 mRNA IRES, a Rhopalosiphum padi virus IRES, a cationic amino acid transporter mRNA IRES, a human insulin-like growth factor II leader 2 mRNA IRES, a giardiavirus IRES, a Smad5 mRNA IRES, a porcine teschovirus-1 talfan IRES, a *Drosophila* Hairless mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2 mRNA IRES, an Epstein-Barr virus IRES, a hibiscus chlorotic ringspot virus IRES, a rat pituitary vasopressin V1b receptor mRNA IRES, and a human hsp70 mRNA IRES.

204. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 203, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to a picornavirus internal ribosome entry site.

205. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 204, wherein the sequence complementary to a picornavirus internal ribosome entry site comprises the sequence:

TTATCATCGTGTTTTTCAAAGGAAAACCACGTCCCCGTGGTTCGGGGGGCCTAGACG
TTTTTTTAACCTCGACTAAACACATGTAAAGCATGTGCACCGAGGCCCCAGATCAGA
TCCCATACAATGGGGTACCTTCTGGGCATCCTTCAGCCCCTTGTTGAATACGCTTGA
GGAGAGCCATTTGACTCTTTCCACAACTATCCAACTCACAACGTGGCACTGGGGTTG
TGCCGCCTTTGCAGGTGTATCTTATACACGTGGCTTTTGGCCGCAGAGGCACCTGTC
GCCAGGTGGGGGGTTCCGCTGCCTGCAAAGGGTCGCTACAGACGTTGTTTGTCTTCA

AGAAGCTTCCAGAGGAACTGCTTCCTTCACGACATTCAACAGACCTTGCATTCCTTT
GGCGAGAGGGGAAAGACCCCTAGGAATGCTCGTCAAGAAGACAGGGCCAGGTTTCC
GGGCCCTCACATTGCCAAAAGACGGCAATATGGTGGAAAATCACATATAGACAAAC
GCACACCGGCCTTATTCCAAGCGGCTTCGGCCAGTAACGTTAGGGGGGGGGGAGGG
AGAGGGGCGGAATT (SEQ ID NO: 6).

206. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 196, wherein the 3' UTR of a positive strand single-stranded RNA virus is a 3' UTR of a positive strand single-stranded RNA virus having no DNA stage.

207. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 206, wherein the 3' UTR of a positive strand single-stranded RNA virus having no DNA stage is a 3' UTR of a bromovirus.

208. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 207, wherein the 3' UTR of a bromovirus is a 3' UTR of a Cowpea chlorotic mottle virus.

209. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 208, wherein a DNA copy of the 3' UTR of a Cowpea chlorotic mottle virus comprises the sequence:

AGTGCCCGCTGAAGAGCGTTACACTAGTGTGGCCTACTTGAAGGCTAGTTATAACCG
TTTCTTTAAACGGTAATCGTTGTTGAAACGTCTTCCTTTTACAAGAGGATTGAGCTGC
CCTTGGGTTTTACTCCTTGAACCCTTCGGAAGAACTCTTTGGAGTTCGTACCAGTACC
TCACATAGTGAGGTAATAAGACTGGTGGGCAGCGCCTAGTCGAAAGACTAGGTGAT
CTCTAAGGAGACC (SEQ ID NO: 8).

210. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 196, further comprising a sequence complementary to an intron.

211. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 196, further comprising a transcription termination signal.

212. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 196, wherein the at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation comprises a recombination site.

213. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 212, wherein the recombination site is selected from the group consisting of a bacteriophage lambda *att* site and a topoisomerase I-based recombination site.

214. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 196, wherein the at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation comprises at least one restriction enzyme recognition site.

215. The method for making a transgenic vector for expression of a heterologous polypeptide in a transgenic cell of claim 196, wherein the at least one restriction enzyme recognition site comprises a polylinker.

216. A kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell, the kit comprising a DNA molecule for construction of a vector for expressing a

heterologous polypeptide in a transgenic cell, the DNA molecule comprising a promoter operably linked, in the 5' to 3' direction, to a DNA sequence comprising:

a) at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation;

b) a sequence complementary to an internal ribosome entry site; and

c) a 3' UTR of a positive strand single-stranded RNA virus.

217. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 216, wherein the promoter is a selected from the group consisting of a constitutive promoter and an inducible promoter.

218. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 217, wherein the promoter is a constitutive promoter.

219. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 218, wherein the constitutive promoter is a eukaryotic constitutive promoter selected from the group consisting of a cauliflower mosaic virus 35S promoter, a blueberry red ringspot virus promoter, a ubiquitin gene promoter, an actin gene promoter, an NeIF-4A10 promoter, a maize Adh1-based pEmu promoter, a barley leaf thionin BTH6 promoter, a cassava vein mosaic virus promoter, a sugarcane bacilliform badnavirus promoter and a histone gene promoter.

220. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 219, wherein the eukaryotic constitutive promoter is a cauliflower mosaic virus 35S promoter.

221. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 220, wherein the cauliflower mosaic virus 35S promoter comprises the

sequence:

AGATTAGCCTTTTCAATTTCAGAAAGAATGCTAACCCACAGATGGTTAGAGAGGCTT
ACGCAGCAGGTCTCATCAAGACGATCTACCCGAGCAATAATCTCCAGGAAATCAAA
TACCTTCCCAAGAAGGTTAAAGATGCAGTCAAAAGATTCAGGACTAACTGCATCAA
GAACACAGAGAAAGATATATTTCTCAAGATCAGAAGTACTATTCCAGTATGGACGA
TTCAAGGCTTGCTTCACAAACCAAGGCAAGTAATAGAGATTGGAGTCTCTAAAAAG
GTAGTTCCCACTGAATCAAAGGCCATGGAGTCAAAGATTCAAATAGAGGACCTAAC
AGAACTCGCCGTAAAGACTGGCGAACAGTTCATACAGAGTCTCTTACGACTCAATG
ACAAGAAGAAAATCTTCGTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAA
AATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAG
GGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTG
AAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAA
GGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCA
CGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGAT
TGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAA
GACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGAACACG (SEQ ID NO: 3).

222. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 216, wherein the coding sequence for a heterologous polypeptide encodes a polypeptide selected from the group consisting of a hormone, an enzyme, a cell toxin, a viral polypeptide, a cell surface polypeptide, and an intracellular polypeptide.

223. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 216, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to an IRES selected from the group consisting of a

picornavirus IRES, a foot-and-mouth disease virus IRES, an encephalomyocarditis virus IRES, a hepatitis A virus IRES, a hepatitis C virus IRES, a human rhinovirus IRES, a poliovirus IRES, a swine vesicular disease virus IRES, a turnip mosaic potyvirus IRES, a human fibroblast growth factor 2 mRNA IRES, a pestivirus IRES, a Leishmania RNA virus IRES, a Moloney murine leukemia virus IRES a human rhinovirus 14 IRES, an aphthovirus IRES, a human immunoglobulin heavy chain binding protein mRNA IRES, a *Drosophila* Antennapedia mRNA IRES, a human fibroblast growth factor 2 mRNA IRES, a hepatitis G virus IRES, a tobamovirus IRES, a vascular endothelial growth factor mRNA IRES, a Coxsackie B group virus IRES, a c-myc protooncogene mRNA IRES, a human MYT2 mRNA IRES, a human parechovirus type 1 virus IRES, a human parechovirus type 2 virus IRES, a eukaryotic initiation factor 4GI mRNA IRES, a Plautia stali intestine virus IRES, a Theiler's murine encephalomyelitis virus IRES, a bovine enterovirus IRES, a connexin 43 mRNA IRES, a homeodomain protein Gtx mRNA IRES, an AML1 transcription factor mRNA IRES, an NF-kappa B repressing factor mRNA IRES, an X-linked inhibitor of apoptosis mRNA IRES, a cricket paralysis virus RNA IRES, a p58(PITSLRE) protein kinase mRNA IRES, an ornithine decarboxylase mRNA IRES, a connexin-32 mRNA IRES, a bovine viral diarrhea virus IRES, an insulin-like growth factor I receptor mRNA IRES, a human immunodeficiency virus type 1 gag gene IRES, a classical swine fever virus IRES, a Kaposi's sarcoma-associated herpes virus IRES, a short IRES selected from a library of random oligonucleotides, a Jembrana disease virus IRES, an apoptotic protease-activating factor 1 mRNA IRES, a Rhopalosiphum padi virus IRES, a cationic amino acid transporter mRNA IRES, a human insulin-like growth factor II leader 2 mRNA IRES, a giardiavirus IRES, a Smad5 mRNA IRES, a porcine teschovirus-1 talfan IRES, a *Drosophila* Hairless mRNA IRES, an hSNM1 mRNA IRES, a Cbfa1/Runx2 mRNA IRES, an Epstein-Barr

virus IRES, a hibiscus chlorotic ringspot virus IRES, a rat pituitary vasopressin V1b receptor mRNA IRES, and a human hsp70 mRNA IRES.

224. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 223, wherein the sequence complementary to an internal ribosome entry site is a sequence complementary to a picornavirus internal ribosome entry site.

225. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 224, wherein the sequence complementary to a picornavirus internal ribosome entry site comprises the sequence:

TTATCATCGTGTTTTTCAAAGGAAAACCACGTCCCCGTGGTTCGGGGGGCCTAGACG
TTTTTTTAACCTCGACTAAACACATGTAAAGCATGTGCACCGAGGCCCCAGATCAGA
TCCCATACAATGGGGTACCTTCTGGGCATCCTTCAGCCCCTTGTTGAATACGCTTGA
GGAGAGCCATTTGACTCTTTCCACAACTATCCAACTCACAACGTGGCACTGGGGTTG
TGCCGCCTTTGCAGGTGTATCTTATACACGTGGCTTTTGGCCGCAGAGGCACCTGTC
GCCAGGTGGGGGGTTCCGCTGCCTGCAAAGGGTCGCTACAGACGTTGTTTGTCTTCA
AGAAGCTTCCAGAGGAACTGCTTCCTTCACGACATTCAACAGACCTTGCATTCCTTT
GGCGAGAGGGGAAAGACCCCTAGGAATGCTCGTCAAGAAGACAGGGCCAGGTTTCC
GGGCCCTCACATTGCCAAAAGACGGCAATATGGTGGAAAATCACATATAGACAAAC
GCACACCGGCCTTATTCCAAGCGGCTTCGGCCAGTAACGTTAGGGGGGGGGGAGGG
AGAGGGGCGGAATT (SEQ ID NO: 6).

226. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 216, wherein the 3' UTR of a positive strand single-stranded RNA virus is a 3' UTR of a positive strand single-stranded RNA virus having no DNA stage.

227. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 226, wherein the 3' UTR of a positive strand single-stranded RNA virus having no DNA stage is a 3' UTR of a bromovirus.

228. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 227, wherein the 3' UTR of a bromovirus is a 3' UTR of a Cowpea chlorotic mottle virus.

229. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 228, wherein a DNA copy of the 3' UTR of a Cowpea chlorotic mottle virus comprises the sequence:

AGTGCCCGCTGAAGAGCGTTACACTAGTGTGGCCTACTTGAAGGCTAGTTATAACCG
TTTCTTTAAACGGTAATCGTTGTTGAAACGTCTTCCTTTTACAAGAGGATTGAGCTGC
CCTTGGGTTTTACTCCTTGAACCCTTCGGAAGAACTCTTTGGAGTTCGTACCAGTACC
TCACATAGTGAGGTAATAAGACTGGTGGGCAGCGCCTAGTCGAAAGACTAGGTGAT
CTCTAAGGAGACC (SEQ ID NO: 8).

230. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 216, further comprising a sequence complementary to an intron.

231. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 216, further comprising a transcription termination signal.

232. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 216, wherein the at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation comprises a recombination site.

233. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 232, wherein the recombination site is selected from the group consisting of a bacteriophage lambda *att* site and a topoisomerase I-based recombination site.

234. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 216, wherein the at least one site for insertion of a sequence comprising coding sequence of a heterologous polypeptide in an antisense orientation comprises at least one restriction enzyme recognition site.

235. The kit for constructing a vector for expressing a heterologous polypeptide in a transgenic cell of claim 216, wherein the at least one restriction enzyme recognition site comprises a polylinker.

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US04/021451

International filing date: 02 July 2004 (02.07.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/485,073
Filing date: 03 July 2003 (03.07.2003)

Date of receipt at the International Bureau: 11 October 2004 (11.10.2004)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse